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THE BIOLOGICAL ACTIVITY OF ARSENOSOBENZENES IN RELATION TO THEIR STRUCTURE*

HARRY EAGLE AND GEORGE O. DOAK

Section on Experimental Therapeutics, Microbiological Institute, National Institutes of Health,† Bethesda 14, Maryland and V. D. Research Laboratory,‡ University of North Carolina, Chapel Hill, N. C.

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I. GENERAL CONSIDERATIONS

Three types of organic compounds containing arsenic linked directly to carbon have been used successfully in the treatment of spirochetal and protozoal diseases: the pentavalent arsonic acids ($RAsO_3H_2$), the arseno compounds (R-As═AsR′), and the arsenoso compounds (RAsO) (or their dithiol derivatives). In these compounds R is usually benzenoid, although similar compounds with a heterocyclic ring, *e.g.*, pyridine, are known to be actively trypanocidal (15). Polycyclic, aliphatic and alicyclic arsenicals have also been tested, but are generally either only weakly active or inert (19, 87).

An important therapeutic consideration is whether the compounds are active as such or must first be modified in the animal host. The classic studies of Ehr-

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* Presented in part at the Chemical-Biological Coordination Center Symposium conducted under the auspices of the National Research Council at the National Academy of Sciences, Washington, D. C., May 26, 1950.

† Public Health Service, Federal Security Agency.

‡ Venereal Disease Division, PHS, FSA.

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I. GENERAL CONSIDERATIONS

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An important therapeutic consideration is whether the compounds are active as such or must first be modified in the animal host. The classic studies of Ehrlich (53, 54) showed the arsonic acids to be highly active therapeutically, but inactive *in vitro*. He concluded (55) that they were reduced to an active trivalent form by the body tissues; the arseno and arsenoso compounds were in fact found to be directly active *in vitro*. The arsenoso compounds were discarded because of their high toxicity. The arseno compounds, notably arsphenamine and neoarsphenamine, found wide therapeutic application.

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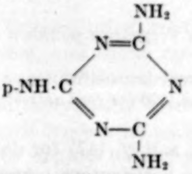
There has, however, been some difference of opinion as to whether the arseno compounds are active as such. Voegtlin and Smith (122) demonstrated in 1920 that after the injection of arsonic acids or arseno compounds there was a latent period before trypanosomes disappeared from the blood of infected animals, while arsenoso compounds produced an immediate and striking decrease. These results were interpreted to mean that the arsenoso compounds were directly trypanocidal, while the arsonic acids had to be reduced and the arseno compounds oxidized to the arsenoso form in the animal body before becoming active. Other workers, however, found that the arsphenamines as such, and without further modification in the animal body, were active against trypanosomes and spirochetes (101, 102, 129). The discrepancy was resolved by later *in vitro* studies with *Treponema pallidum* (32). Although the arsphenamines were apparently quite active *in vitro* against this organism when tested by the usual anaerobic technic, when both the dissolution and testing of the drugs were carried out under an atmosphere of nitrogen, their activity was strikingly reduced. The arsphenamines as such are therefore not directly treponemicidal, but in the course of dissolution and dilution prior to testing there is sufficient oxidation to the arsenoso compound to make the solution active. In general, the arseno compounds are amorphous powders which are almost impossible to obtain in a state of chemical purity, and exist in several states of molecular aggregation. Both factors have been shown to influence their parasiticidal activity and toxicity (93, 128).

The fact that the arsonic acids and arseno compounds must be modified in the animal body before they exert their therapeutic action prevents the correlation of their chemical structure with biological activity unless all the compounds are modified *in vivo* to the same degree. This assumption is certainly not justified in the case of the arsonic acids which are excreted in large part unchanged. Cohen, King and Strangeways (23) studied the rate of oxidation of a number of arsenoso compounds, on the assumption that this might be a measure of the rate of reduction of the corresponding arsonic acids *in vivo*, and thus of their toxicity. No such correlation was found. Similarly, in later studies from our laboratory (47) there was no regular correlation between the toxicity (or trypanocidal activity) of arsonic acids and that of the corresponding arsenosobenzenes (Table 1).

The arsenoso compounds therefore appear to be the drugs of choice in an attempted correlation of chemical structure and biological activity. Unfortunately for that purpose, most pharmacological studies on the aromatic arsenicals have dealt with arsonic acids and arseno compounds, on the assumption that the arsenoso compounds were too toxic for therapeutic use. This assumption was questioned by Tatum and Cooper (114) who demonstrated that m-amino-p-hydroxy-phenylarsenoxide, which they named "mapharsen" (oxophenarsine hydrochloride) possessed a higher therapeutic index against *Treponema pallidum* than the arsphenamines. Prior to the advent of penicillin, this compound was widely regarded as the drug of choice for the treatment of syphilis. A large series of arsenoso compounds were later found to possess a higher therapeutic index than the corresponding arsonic acids against *T. equiperdum* (47), although Gough and King had previously reported no essential difference between the two classes of compounds in a smaller series (69).

TABLE 1

The poor correlation between (A) the toxicities of arsonic acid and the corresponding arsenoso compounds, and (B) the trypanocidal activities in vivo *of arsonic acids and the corresponding arsenoso compounds. (Summarized from 40, 41, 46, 47)*

SUBSTITUENT GROUP	A. TOXICITY IN MICE			B. TRYPANOCIDAL ACTIVITY IN MICE (*T. equiperdum*)		
	LD$_{50}$		Ratio of LD$_{50}$	CD$_{50}$		Ratio of CD$_{50}$
	As^{V}	As^{III}		As^{V}	As^{III}	
	*mg./kg.**			*mg./kg.**		
p-$CONHCH_2CONH_2$	1750	15	115	380	1.4	270
p-$NHCH_2CONH_2$	950	19	50			
p-$NHCONH_2$	1025	11	93	305	1.16	267
p-OCH_2CONH_2	745	9.5	78	80	0.82	98
3-$NHCOCH_3$-4-OH	765	6	127	565	1.91	296
3-NH_2-4-OH	775	17	46	85	0.62	137
p-SO_2NH_2	595	18	33	170	1.0	85
p-OH	550	2	275			
"Melarsen" 	260	12	22	30	0.048	625
3-NH_2-4-$CONH_2$	290	15	19			
3-NH_2-4-$(CH_2)_2COOH$	140	12	12	>190	1.26	>150
p-NH_2	165	1.5	110			

* mg./kg. of As

The present paper will consider the toxicity and parasiticidal activity of arsenosobenzenes in relation to the substituent groups on the benzene ring, preliminary to a discussion of their mode of action. Although attention has focussed largely on the mono-substituted compounds (28–31), a number of di-substituted derivatives of arsenosobenzene have also been prepared (22) (30, 86). In addition to the derivatives of arsenosobenzene, a limited number of arsenoso compounds with naphthalene, biphenyl, pyridine and similar ring structures have been studied. Finally, a number of thioarsenites, compounds formed by the condensation of arsenosobenzenes with various thiol compounds, have been tested for toxicity and parasiticidal activity in a number of protozoal infections.

Toxicity has usually been determined as the 50 per cent lethal dose on single intraperitoneal injection in white mice (34), but has in some studies been determined also in rats, rabbits (47) and dogs. In general, the relative toxicity of the compounds in mice closely paralleled their relative toxicity in rabbits (Fig. 1). Parasiticidal activity has been studied with *Treponema pallidum* (34) and *Trypanosoma equiperdum* (47), both *in vitro* and *in vivo;* a few compounds have further been tested against *Trypanosoma cruzi* (38, 39), *Wuchereria bancrofti* (82, 116), *Litomosoides carinii* (88, 89), *Endamoeba histolytica* (2–6), and *Leishmania donovani* (39).

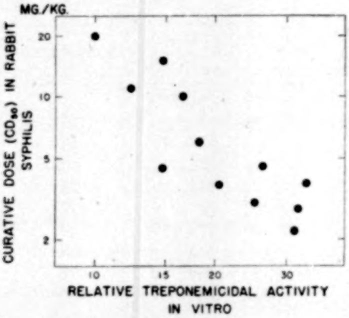
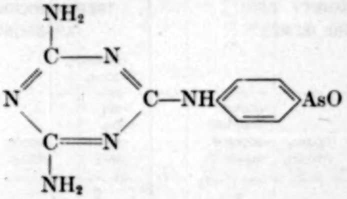


FIG. 1. Correlation between activity of arsenosobenzenes on *Treponema pallidum in vivo* and *in vitro*. (After 34, 40, 41, 46.)

Relative treponemicidal activity *in vitro* determined by direct immobilization; the unsubstituted arsenosobenzene is used as the reference compound (100 per cent activity).

In some infections (*cf.* Fig. 1 in re syphilitic infection, and *cf.* (47) for data in experimental trypanosomiasis) there was a reasonably satisfactory correlation between the relative parasiticidal action of a series of compounds *in vitro* and their therapeutic action *in vivo*. With other organisms, however, there was no correlation whatever between the direct parasiticidal action of arsenosobenzenes *in vitro* and their therapeutic action *in vivo*. With both *L. donovani* and *Trypanosoma cruzi*, a number of compounds which were highly active when added directly to the cultured flagellate proved wholly ineffective in the treatment of hamster infections (38, 39). The failure of the drug in these two instances may be related in part to the fact that in both infections the parasite in the infected host is in a different stage of its life cycle and differs morphologically and perhaps metabolically from the cultured organisms. Further, in both infections the organisms localize inside host cells, within which the drug may not attain the same concentrations as in extracellular fluids. Another example of the importance of the permeability factor *in vivo* is the relative ineffectiveness of the arsenobenzenes in the treatment of central nervous system syphilis or trypanosomiasis. Even compounds which are highly effective in the treatment of the early stages of the disease may be relatively ineffective in cases with central nervous system involvement.

When a number of arsenosobenzenes were tested for therapeutic activity in both mouse and rabbit trypanosomiasis, there was generally good agreement in their relative activity in the two host species (47). An exception was, however, noted in the case of "melarsen oxide"



(59–63). The trypanocidal activity (*T. equiperdum*) of this compound in mice (CD_{50} = 0.2 mg./kg.) greatly exceeded that in rabbits (CD_{50} = 10 mg./kg.), and was far greater than would have been indicated by its trypanocidal activity *in vitro* (39). This peculiarly enhanced parasiticidal activity in mice deserves further study.

II. THE BIOLOGICAL ACTIVITY OF SUBSTITUTED ARSENOSOBENZENES

A. *Activity in relation to structure*

Unsubstituted arsenosobenzene is highly toxic for every cell species so far tested, and shows no evidence of specificity in its cytotoxic effect. Since it is as toxic for the mammalian host as for the parasite, the problem of finding therapeutically useful arsenosobenzenes becomes one of finding substituent groups which depress the toxicity of the compound for the host to a greater extent than they depress its parasiticidal action.

The effect of a number of single substituents on the toxicity and direct treponemicidal (*T. pallidum*) activity of arsenosobenzene is shown in Fig. 2 and Table 2. The latter also shows their direct trypanocidal activity (*Trypanosoma equiperdum*). Despite the wide variations encountered between closely related compounds, the data permit some tentative generalizations with respect to the correlation between chemical structure and biological activity in this series of compounds.

1. *"Inert" substituents.* There were a number of "inert" substituents (*e.g.*, —Cl, —NO_2, —NH_2, —OH, —CH_3, —F) which did not significantly affect either the toxicity or parasiticidal activity of arsenosobenzene. The activity: toxicity ratios of the resulting compounds were therefore substantially the same as that of the highly active and highly toxic parent arsenosobenzene. Like the latter, these compounds apparently have no selective or specific effect on a particular parasite. The position of such substituents on the benzene ring also had relatively little effect. Thus, the ortho-, meta- and para-arsenosotoluene com-

pounds had relative treponemicidal activities of 84, 97 and 102, respectively (referred to the parent arsenosobenzene as 100), relative toxicities of 88, 100 and 118, and activity:toxicity ratios of 0.95, 0.98 and 0.83.

2. *Acidic substituents.* Most acidic substituents markedly depressed both the treponemicidal and trypanocidal activity of arsenosobenzene, and resulted in compounds with a low level of activity (*cf.* Fig. 2 and Tables 2 and 3). The toxicity of these compounds was also reduced, but usually not to the same degree as the parasiticidal activity. In consequence, the parasiticidal activity:toxicity ratios of the acid-substituted compounds were even less than that of the parent compound. There were, however, a few important exceptions to this generalization, notably the γ-(p-arsenosophenyl)-butyric acid-, δ-(p-arsenosophenyl)-valeric acid-, and ε-(p-arsenosophenyl)-caproic acids (*cf.* Table 3). The first two compounds were actively trypanocidal against *Trypanosoma equiperdum*, both *in vivo* and *in vitro*, although they had no significant activity against *T. pallidum*. Conversely, the last-named compound had considerable treponemicidal activity, but was almost inert against *Trypanosoma equiperdum*. These exceptions emphasize the danger of generalizing even on the basis of a large number of closely

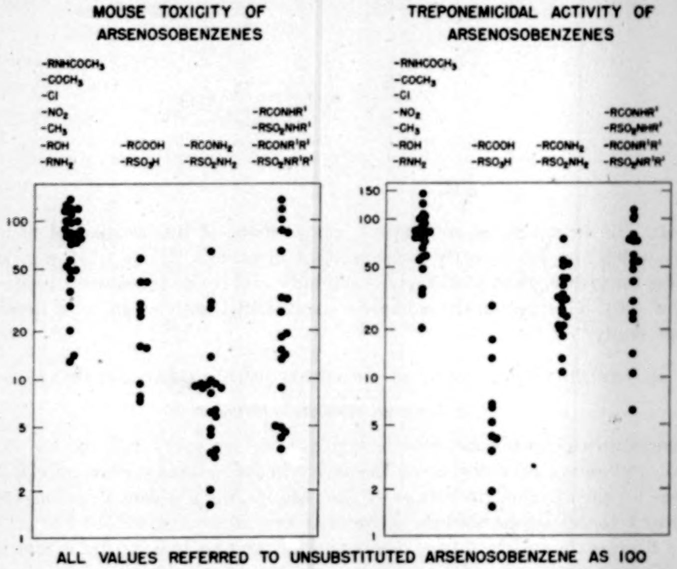


FIG. 2. The effect of various types of single substituents on the toxicity and treponemicidal activity of representative arsenosobenzenes. (After 41, 46, 47.)

Treponemicidal activity determined by direct immobilization *in vitro;* the unsubstituted arsenosobenzene is used as the reference compound.

TABLE 2

The effect of various single substituents on the toxicity and the treponemicidal and trypanocidal activity of arsenosobenzene

(All values referred to that of unsubstituted arsenosobenzene as 100.) (Summarized from 40, 41, 46, 47)

SUBSTITUENTS		TOXICITY IN MICE*	TREPONEMICIDAL ACTIVITY *in vitro** (*Trep. pallidum*)	TRYPANOCIDAL ACTIVITY *in vitro** (*T. equiperdum*)	RATIO OF TREPONEMICIDAL ACTIVITY *in vitro*: TOXICITY	RATIO OF TRYPANOCIDAL ACTIVITY *in vitro*: TOXICITY
"Inert"	NO_2, $—CH_3$, —Cl $—NH_2$, —OH, —F	(17) 36–123 85	(17) 36–147 85	(7) 57–102 91	0.33–1.47 1.0	0.80–1.2 0.92
"Slightly active"	$—RNH_2$, —ROH, $—NHCOCH_3$, $R—NHCOCH_3$, $—OCOCH_3$	(12) 6.7–76 23	(12) 21–78 38	(6) 31–71 34	0.38–4.8 1.76	1.2–6.0 2.2
Acidic (*cf.* Table 3)	—COOH, $—SO_3H$, —RCOOH	(15) 5.8–41 16	(14) 0.7–28 4.7	(12) 0.06–54 3.0	0.04–2.7 0.24	0.002–6.1‡ 0.1
Acid amides† (*cf.* Table 5)	$—CONH_2$, $—SO_2NH_2$, $—RCONH_2$	(16) 3.2–14 7.2	(16) 11–52 30	(12) 1.4–73 25	2.3–6.0 4.5	0.4–7.4 3.8

* ( ) = no. of compounds tested.
36–123 = range.
85 = median.

† Substituted amides not included.

‡ Anomalous result; next highest ratio was 0.9; *cf.* Table 3.

related compounds. They emphasize also the specificity of the reaction of some arsenoso compounds with particular parasites (*cf.* page 132).

The para-arsenosophenylbutyric acid compound has proved highly effective in the treatment of early human trypanosomiasis (*Trypanosoma gambiense*) (35, 37). It has proved effective also against so-called arsenic-resistant strains of that species (35, 37, 47, 48, 118) (*cf.* page 136). In the late forms of the infection, however, after the central nervous system has been invaded, it has proved relatively ineffective, presumably because the compound does not there attain actively trypanocidal concentrations. Its possible use in conjunction with tryparsamide in these late manifestations is currently under study. In loa-loa, although the drug caused a temporary disappearance of micro-filaria from the blood stream, no permanent cure was effected (56). Corresponding to the results obtained in experimental mouse infections with *Trypanosoma cruzi* (cf. page 124), the compound proved ineffective in the treatment of the human infection (113). In surra (*Trypanosoma evansi*), Mace, Ott and Cortez (80) have reported that, although the drug has some measure of trypanocidal activity, cure is effected in only a small proportion of the animals treated at subtoxic levels of

TABLE 3

The relative molar toxicity and parasiticidal activity of acid-substituted phenyl arsenoxides. (After 36, 39, 44, 47).

COMPOUND	pK	TOXICITY*	RELATIVE ACTIVITY IN VITRO AGAINST:			
			Trep. pallidum	*Tryp. equiperdum*	*Trypanosoma cruzi*	*L. donovani*
Unsubstituted phenyl arsenoxide	—	100	100	100	100	100
o-SO_3H	2	5.7	1.5	0.02	0.02	0.02
p-COOH	4.0	41	6.7	0.45	0.04	0.21
p-CH_2COOH	4.35	41	4.2	4.7		
p-$(CH_2)_2COOH$	4.7	7.3	4.1	2.8		
p-$(CH_2)_3COOH$	4.9	8.7	1.9	54		32
p-$(CH_2)_4COOH$	5.23	7.4	—	27	47	30
p-$(CH_2)_5COOH$	5.35±	8.1	22	7.5	12	2.5
p-COOH	4.0	41	6.7	0.45	0.04	0.21
m-COOH	4.25	16	13	—	0.3	1.6
o-COOH	5.55	27	28	3.2		1.5±
3-NO_2-4-COOH	2.6	27	18	17	24	40±
p-OCH_2COOH	3.3	25	4.2	4.5	0.3	6.8
3-NH_2-4-COOH	4.7	15	20	4.0	0.05	0.12
p-NHCO$(CH_2)_2COOH$	4.75	7.7	6.4	0.4±		0.15±
3-NH_2-4-$(CH_2)_3COOH$	5.34	7.2	—	26	62	26

* Single intraperitoneal injection in white mice. All values referred to that of phenyl arsenoxide as 100.

the drug. The results obtained with p-arsenosophenylbutyric acid in a number of experimental trypanosomal infections are summarized in Table 4.

a. *Evidence for modification of acid-substituted arsenoso compounds in vivo.* There is reason to believe that with these acidic compounds the observed toxicity is not that of the compound itself but of a derivative formed in the body. 1) As is discussed in a following section, the toxicity of arsenicals is apparently determined by the degree to which they are bound by the host tissues (73). There was a high degree of correlation between the rate of excretion of an arsenical and its toxicity, the least toxic compounds being excreted at the fastest rate. Further, the degree to which arsenoso compounds were bound by red cells *in vitro* proved a generally reliable measure of their systemic toxicity. The acid-substituted compounds were bound to only a negligible degree by erythrocytes *in vitro;* correspondingly, after intravenous injection they were at first excreted rapidly. However, after a few hours, the rate of excretion fell off abruptly, which suggested that the arsenical had been modified to a more toxic form which was then retained in the body. 2) After lethal doses of acid-substituted compounds, death was usually delayed as compared with similarly toxic doses of other arsenoso compounds not containing acidic groups (Fig. 3).

TABLE 4

Trypanocidal activity of γ-para-arsenosophenylbutyric acid in vivo

TRYPANOSOMAL SPECIES	HOST ANIMAL	TOTAL CD_{50}	REFERENCES
		mg./kg.	
T. equiperdum	Mouse	3.4[*]	47
	Rabbit	6.0[†]	47
T. gambiense	Guinea pig	4	119
	Man	7[‡]	35, 37
T. rhodesiense	Mice	3	17
		7	
T. congolense	Mice	No effect	17, 26
	Cattle	No effect	39
T. evansi	Mules and horses	7.5±[§]	24
	Rats and dogs	Recurrence prevented in 50% of animals only at toxic dosages. (10 doses at 3.5–5 mg./kg. each)	80
T. cruzi	Rats	Only temporary disappearance of organisms from blood	39
	Mice		26

[*] Single intraperitoneal injection.
[†] 4 daily intravenous injections.
[‡] 7 to 14 daily intravenous injections.
[§] 6 injections.

These several lines of evidence indicate that the acidic group may be modified in the animal body, possibly by esterification or some other type of conjugation, to form a more toxic arsenical. The ordinary body mechanisms for defense against organic acids in this particular case apparently increase the reactivity of the compound with the tissues, and increase rather than decrease its toxicity.

b. *The effect of pH on the parasiticidal effect of arsenoso compounds.* There is reason to believe that such slight parasiticidal activity as acid-substituted arsenoso compounds possess is largely a function of the non-ionized molecule, and that the ion is usually relatively inactive. In preliminary studies there was a rough correlation between the pK of the acid and its treponemicidal activity *in vitro* (44). The effect of pH on both treponemicidal and trypanocidal action was therefore studied in a larger series of compounds (36).

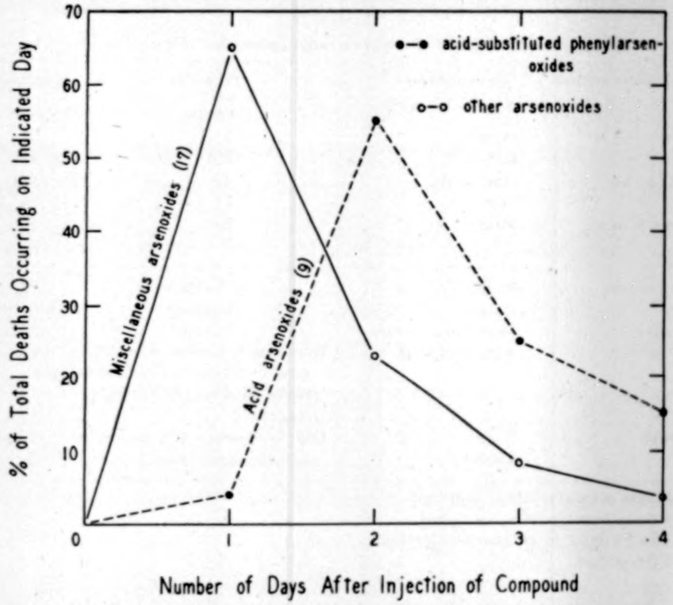


FIG. 3. The characteristic delay in the death of white mice injected intraperitoneally with LD_{40} to LD_{90} of acid-substituted arsenosobenzenes. (After 73.)

The parasiticidal activity of arsenosobenzene and of derivatives containing non-acid substituents was independent of the pH of the testing medium over the total range of viability of the organism. With acid-substituted compounds, however, the activity against both *Trypanosoma equiperdum* and *Treponema pallidum* usually increased strikingly with the hydrogen ion concentration (Fig. 4). This effect of pH was referable to the fact that the ionized salts of the acid-substituted compounds were relatively inactive as compared with the undissociated acid. The activity changed with pH in inverse relation to the degree of ionization, and was indeed predictable from the pK of the acidic group. With strong acids, such as the p-SO_3H compound (pK = 2±), there was no demonstrable change in activity in the range pH 8.5–5.5, throughout which the compound would be essentially completely ionized. With weak acids, however, the activity varied as much as a hundred-fold over the range pH 8.5–5.5 (*cf.* right hand portion of Fig. 4).

With several of the acids, the ionized form, while less active than the undissociated molecule, nevertheless had a definite parasiticidal action. With such compounds, the total parasiticidal activity at a given pH was therefore a sum of the (slight) activity of the ionized fraction and the activity of the non-dissociated free acid, the proportion of the latter at a given pH depending on the pK of the compound. Thus, the ions of the p-$(CH_2)_3COOH$ and 3-NO_2-4-COOH arsenosobenzenes had an unusually high trypanocidal activity which contributed significantly to the total activity noted at, *e.g.*, pH 7.0.

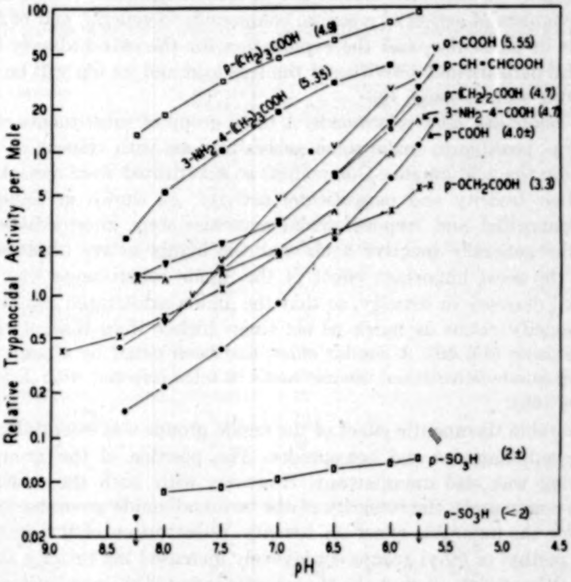


FIG. 4. The effect of pH on the trypanocidal activity of acid-substituted arsenosobenzenes. (After 36.)

Figures given in parentheses are the pK′ values of the various acids.

One may therefore conclude that in a series of acid-substituted arsenoso compounds, the most effective against a given organism would be that compound with the highest pK (*i.e.*, with the largest proportion in undissociated form at body pH), the ion of which was also moderately active against the parasite. In a series of compounds with substituent groups R—COOH or R—SO_3H, some interest therefore attaches to the effect of the radical R on the dissociation constant of the terminal acidic group (*cf.* Table 3). In the series —$(CH_2)_nCOOH$, there was a progressive increase of 0.25–0.35 units in the pK of the compound with each additional methylene group; an extension of the series might disclose compounds of therapeutic interest.

The high activity *in vitro* of some of these acid-substituted compounds against cultures of *Trypanosoma cruzi* and *Leishmania donovani* is particularly to be noted. They were, however, not effective in the treatment of the experimental infections, perhaps because the intracellular localization of the parasites in the infected animal rendered them inaccessible to the drug. In addition, preliminary data (39) indicate that the susceptibility of the intracellular parasites to arsenoso compounds may be less than that of the cultured flagellate.

The mechanism of action of arsenoso compounds in general, and of these acid compounds in particular, and the explanation for the extraordinary difference between the parasiticidal activities of the free acid and its ion will be discussed in a following section (page 133).

3. *Acid-amide and ester substituents.* A third group of substituents concerning which it was possible to make some generalizations with respect to biological activity were the acid amides. Compounds so substituted were remarkably uniform in their toxicity and parasiticidal activity. As shown in Table 5, their direct trypanocidal and treponemicidal activities were intermediate between those of the generally inactive acids and the highly active arsenosobenzene. However, the most important effect of the amide substituents was a regular and marked decrease in toxicity, so that the amide-substituted compounds had activity:toxicity ratios as much as six times higher than that of the parent arsenosobenzene (45, 46). A similar effect had been noted by Gough and King in studying amide-substituted arsonic acids in mice infected with *Trypanosoma equiperdum* (69).

The favorable therapeutic effect of the amide groups was essentially the same with both sulfonamides and benzamides. The position of the groups on the benzene ring was also unimportant. However, with both the p-$CONH_2$ and p-SO_2NH_2 compounds, the integrity of the terminal amide grouping was usually essential for the favorable effect on toxicity. Substitution of the amide hydrogens with methyl or ethyl groups successively increased the toxicity at a greater rate than the activity, so that the therapeutic index of these substituted amides was considerably less than that of the amides. The p-SO_2NH_2, p-SO_2NHCH_3 and p-$SO_2N(CH_3)_2$ arsenoso compounds had treponemicidal activities *in vitro* of 29, 72 and 112, relative toxicities of 4.8, 18 and 93, and activity:toxicity ratios of 6.1, 4.0 and 2.3, respectively. Similar effects were obtained on substituting amide hydrogens in the p-$CONH_2$ compound.

In contrast to the $CONHCH_3$ or —$SO_2NHC_2H_5$ compounds, when the terminal grouping was one which was itself "eutherapeutic," *i.e.*, depressed toxicity more than activity, the resulting compound was just as useful as the parent amide. Thus, the $CONHC_2H_4OH$, $CONHC_6H_4NHCOCH_3$ and $SO_2NHC_2H_4OH$ compounds compared favorably with the parent amides with respect to toxicity and parasiticidal activity.

The favorable effect of amide-substitution in depressing the toxicity of phenylarsenoxide to a much greater extent than its parasiticidal activity has been noted in a number of other infections. In cotton rats infected with *Litomosoides carinii* and in dogs infected with *Dirofilaria immitis*, Otto and Maren (88, 89) found a number of trivalent amide-substituted arsenicals to be effective against the adult forms at less than the toxic dosages. Fulton and Yorke (64) had previously commented on the efficacy of reduced tryparsamide (the p-NHCH$_2$CONH$_2$ compound) in *Trypanosoma rhodesiense* infections in mice. The reduced form of carbarsone (p-NHCONH$_2$ arsenosobenzene) has been found to be more actively amebicidal both *in vitro* and *in vivo* than the corresponding arsonic acid, and effective doses were reasonably well tolerated by rabbits, monkeys and man (2, 4).

TABLE 5

The relative molar toxicity and parasiticidal activity of amide-substituted arsenosobenzenes (45–48)

(All values referred to that of the unsubstituted arsenosobenzene as 100)

COMPOUND	RELATIVE TOXICITY IN WHITE MICE	RELATIVE ACTIVITY IN VITRO AGAINST:				
		T. pallidum	*T. equiperdum*	Arsenic-resistant *T. equiperdum*	*T. cruzi*	*L. donovani*
m-CONH$_2$	9.8	41	39	1.75	0.25	0.53
p-CONH$_2$	9.6	45	45	0.34	0.07	0.08
p-CH=CHCONH$_2$	9.7	42	73	—	—	0.98
p-CH$_2$CONH$_2$	8.6	20	31	—	—	—
p-(CH$_2$)$_2$CONH$_2$	13.5	33	60	8.15	8.3	4.5
p-CONHCONH$_2$	6.4	34	34	2.97	0.16	0.08
p-CONHCH$_2$CONH$_2$	3.86	24	15.0	0.68	<0.04	0.09
p-CONHCH$_2$CH$_2$CONH$_2$	3.2	13	—	—	—	—
p-CH$_2$CONHCH$_2$CONH$_2$	3.4	11	1.5	—	<0.08	5.2
p-OCH$_2$CONH$_2$	9.0	52	26	2.02	0.18	4.3
p-NHCONH$_2$	8.1	38	29	0.57	0.2	0.07
p-NHCH$_2$CONH$_2$	4.5	22	—	—	—	—
p-NHCO(CH$_2$)$_2$CONH$_2$	9.0	25	—	—	—	—
m-SO$_2$NH$_2$	6.1	21	7.8	—	—	—
p-SO$_2$NH$_2$	4.8	29	24	0.16	0.03	0.05
p-SO$_2$NHCH$_2$CONH$_2$	3.5	17	1.4	—	—	0.22
p-CONHC$_2$H$_4$OH	4.8	22	39	0.23	0.07	0.05
p-SO$_2$NHC$_2$H$_4$OH	4.2	23	9.0	0.18	—	0.13
p-CONHCH$_2$CN	4.5	27.0	19.5	0.42	0.08±	0.15
3-NH$_2$-4-CONH$_2$	5.6	28	52	2.76	0.06	0.15
3-OH-4-CONH$_2$	23	45	48	—	—	—

(Unlike amide-substitution, the esterification of acid compounds greatly increased both their parasiticidal activity and their toxicity. In consequence, such ester-containing compounds, when stable, were not better therapeutically than the parent arsenosobenzene. Many hydrolyzed in aqueous solution, in which case their biological activity was the same as that of the acid-substituted compound.)

4. *Miscellaneous substituents* ($-RNH_2$, $-NHR$, $-RNHR'$). Highly variable results have been obtained with arsenosobenzenes containing terminal or substituted amino- or hydroxy-groups in a ring substituent. The treponemicidal activity *in vitro* of a number of such compounds referred to that of arsenosobenzene as 100 varied between 3 and 78; their toxicity varied between 6.7 and 76; and their activity:toxicity ratio varied between 0.38 and 2.6 times that of the parent arsenosobenzene. In no instance was a compound obtained which was sufficiently parasiticidal and non-toxic to suggest the possibility of its therapeutic use for the particular infections tested.

An important exception, however, is provided by "melarsen oxide" (p-(2,4-diamino-S-triazinyl-6)-aminoarsenosobenzene) (59–63). This compound is a highly active trypanocidal agent (*cf.* page 111) and has proved effective in the treatment of early cases of the human disease (*T. gambiense*). Earlier reports indicated that like para-arsenosophenylbutyric acid it was ineffective in the late manifestations of the disease (85). A more recent report by Friedheim, however, suggests that it may have an activity in late cases as well (61). The anilino-pyrimidine analagous to this anilino-triazine compound was less actively trypanocidal (7).

5. *The importance of the terminal grouping in a substituent.* Single substituents have thus been shown to have widely varying effects on the toxicity and parasiticidal activity of arsenosobenzene. In a number of the compounds, the substituent was a side chain of varying length. In general, and regardless of the length or nature of the side chain, the activity and toxicity of the compound were usually determined by the nature of the terminal functional groupings. Thus, the $-CH_3$ group had been found to be "inert," while the $-CONH_2$ and $-SO_2NH_2$ groups had a highly favorable effect on toxicity. The p-CH_2CONH_2, $(CH_2)_3CONH_2$ arsenosobenzenes all behaved as amides (Table 4); while in the CO_2NHCH_3 and the $CO_2N(CH_3)_2$ compounds the favorable effect of the amide grouping was diminished or abolished by the substitution of methyl groups for amide hydrogens (*cf.* page 118). Similarly, when an amide hydrogen was replaced by a group carrying a terminal acidic group (*e.g.*, the $CONHCH_2COOH$), the pharmacologic and parasiticidal properties of the compounds were determined by the terminal acidic group and not by the amide.

6. *Multiple substituents* (*41*). The effects of multiple substitution on the toxicity and parasiticidal activity of arsenosobenzene are difficult to interpret. Only a relatively small number of compounds have been tested; in no case could the effect of double substitution be anticipated from the effect of each group alone. Thus, six of the ten possible arsenosoaminophenols were prepared and tested for activity on *T. pallidum in vitro* (Table 6). The $-NH_2$ and $-OH$ substituents separately, whether o-, m-, or -p- to the arsenoso group, had resulted in a uniform series of compounds, with treponemicidal activities varying only from 72 to 98, toxicities from 49 to 85, and activity:toxicity ratios from 1.0 to 1.46. The treponemicidal activity of the six aminophenol compounds was also remarkably uniform, varying only between 39 and 57; but their toxicities varied between 6.94 and 78.9, so that the activity:toxicity ratios varied ten-

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fold, from 0.54 to 5.5. The combination 3-NH$_2$-4-OH which is the well-known compound oxophenarsine ("mapharsen"), was the most favorable combination in the entire series. At the other extreme, the combination 3-NH$_2$ 2-OH gave a less favorable therapeutic index than arsenosobenzene itself. Any change in the 3-NH$_2$-4-OH combination, either by the introduction of a third substituent, extending either group on a side chain, or substitution of the hydrogen in either group, abolished its highly favorable properties.

Similarly variable and unpredictable results were obtained with the arsenosoaminobenzoic acids. Nine of the ten possible isomers were tested for direct trypanocidal activity. Unlike the example of the aminophenols just cited, the trypanocidal activities of these compounds varied 40-fold, from 0.6 to 23, with the 3-NH$_2$-2-COOH compound showing the highest activity (36, 47).

While no combination of two wholly inert substituents (—CH$_3$, —NO$_2$, —Cl, etc.) resulted in compounds with a highly favorable activity: toxicity ratio, one cannot say unequivocally that further search would not reveal such a combination.

TABLE 6

The treponemicidal activity and toxicity of aminophenol-substituted arsenosobenzenes. (*After 41*)

SUBSTITUENT GROUPS	MOLAR TOXICITY	MOLAR TREPONEMICIDAL ACTIVITY	RATIO OF ACTIVITY: TOXICITY
3-NH$_2$-4-OH	6.9	42	6.1
3-OH-4-NH$_2$	10	41	3.9
2-NH$_2$-3-OH	10	34	3.3
2-OH-5-NH$_2$	22	39	1.8
3-OH-5-NH$_2$	74	57	0.78
2-OH-3-NH$_2$	79	43	0.54

Since the favorable therapeutic activity of oxophenarsine in the treatment of syphilis rests primarily on its strikingly reduced toxicity (6.9 per cent of that of the unsubstituted arsenosobenzene in mice and 9.4 per cent in rabbits), it was to be anticipated that it would also be useful in a number of other infections in which it had a direct parasiticidal activity. This has proved to be the case. It was just as effective in the treatment of yaws as in the treatment of syphilis. Although it is definitely parasiticidal against *Borrelia recurrentis* and causes the rapid disappearance of the organisms in mouse infections, its therapeutic activity in the human infection in terms of cure and reduced mortality are questionable. Other workers (116) have reported initially favorable results in the treatment of human filariasis with mapharsen. Although Anderson and Chuan (3) found it to be amebacidal *in vitro*, its therapeutic value in this disease has not been thoroughly studied.

7. *Thioarsenites*. The chemistry and pharmacology of the thioarsenites have been studied by Barber (8), Cohen, King and Strangeways (21), and Strangeways (111), who prepared a number of such compounds and determined their

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efficacy in experimental trypanosomiasis in white mice. Strangeways (111) concluded that their trypanocidal activity, whether *in vitro* or *in vivo*, depended on their hydrolysis to the corresponding arsenoso compound, the reverse of the reaction shown on page 125. Murgatroyd (85) tested two of these compounds (the di-glutathionyl- of 4-NHCOCH$_3$-2-OH-arsenosobenzene (K350) and the di-cysteinyl of 4-CONH$_2$-arsenosobenzene (K352)) in the treatment of *T. gambiense* infections in man. In maximum tolerated doses, they had no effect on central nervous system infections, and relatively small doses were toxic. Eckler and Shonle (51) studied the therapeutic activity of the disodium salt of p-[bis (p-sulfophenylthio)arsino]-acetanilid in rat trypanosomiasis and rabbit syphilis, with results which compared favorably with those obtained with arsphenamine. In the treatment of experimental and human filariasis, Otto and Maren (88, 89) and their coworkers (116) found the dithioglycolate of the 4-CONH$_2$ compound to kill the adult forms of *Dirofilaria immitis* in dogs and of *Litomosoides carinii* in cotton rats, and the microfilaria of *Wuchereria bancrofti* in man, and to have essentially the same activity as the parent arsenosobenzene. In experimental amebiasis, Anderson and his coworkers (2–6) found both the dithioglycolate and dithiosalicylate of reduced carbarsone (p-NHCONH$_2$-arsenosobenzene) to be effective in the treatment of monkeys infected with *Endameba histolytica*. In human cases, Anderson and his coworkers (6) were able to cure 33 of 39 and 41 of 44 cases treated with the dithioglycolate and dithiosalicylate, respectively, at total dosages which varied from seven grams given in a period of 24 hours, to three grams given over a period of 10 days. In human trypanosomiasis (*T. gambiense*) Friedheim (62) has reported initially favorable results with an "alkyl mercapto" derivative of melarsen oxide.

A number of thioarsenites prepared and tested in this laboratory are listed in Table 7. As there shown, they were regularly less toxic but also less actively parasiticidal *in vitro* than the parent arsenosobenzenes. *In vivo* also, whether in the treatment of rabbit or mouse trypanosomiasis, their therapeutic index, expressed in Table 7 as the ratio of $\frac{LD_{50}}{CD_{50}}$, was generally no more favorable and in several instances less favorable than that of the corresponding arsenosobenzene.

Depending on the particular thiol compound used for conjugation, the thioarsenites often have a clear advantage over the corresponding arsenosobenzene in their greater solubility. It has not been proved, however, that they have a more favorable therapeutic index in man than the parent arsenosobenzene, *i.e.*, that their toxicity has been reduced to a greater extent than their parasiticidal activity. In at least some experimental infections, that has not been found to be the case (*cf.* Table 7).

It is an open question whether the activity and toxicity of thioarsenites is determined solely by the degree to which they hydrolyze to the corresponding arsenosobenzene, or whether they may act directly. It is true that an excess of thiol abolishes the parasiticidal activity of an arsenoso compound, both *in vitro* and *in vivo* (*cf.* page 127 et seq.). These data would suggest that in accordance with the suggestion of Gough and King ((69);*cf.* also (21)) the hydrolysis of efficacy in experimental trypanosomiasis in white mice. Strangeways (111) concluded that their trypanocidal activity, whether *in vitro* or *in vivo*, depended on their hydrolysis to the corresponding arsenoso compound, the reverse of the reaction shown on page 125. Murgatroyd (85) tested two of these compounds (the di-glutathionyl- of 4-$NHCOCH_3$-2-OH-arsenosobenzene (K350) and the dicysteinyl of 4-$CONH_2$-arsenosobenzene (K352)) in the treatment of *T. gambiense* infections in man. In maximum tolerated doses, they had no effect on central nervous system infections, and relatively small doses were toxic. Eckler and Shonle (51) studied the therapeutic activity of the disodium salt of p-[bis (p-sulfophenylthio)arsino]-acetanilid in rat trypanosomiasis and rabbit syphilis, with results which compared favorably with those obtained with arsphenamine. In the treatment of experimental and human filariasis, Otto and Maren (88, 89) and their coworkers (116) found the dithioglycolate of the 4-$CONH_2$ compound to kill the adult forms of *Dirofilaria immitis* in dogs and of *Litomosoides carinii* in cotton rats, and the microfilaria of *Wuchereria bancrofti* in man, and to have essentially the same activity as the parent arsenosobenzene. In experimental amebiasis, Anderson and his coworkers (2–6) found both the dithioglycolate and dithiosalicylate of reduced carbarsone (p-$NHCONH_2$-arsenosobenzene) to be effective in the treatment of monkeys infected with *Endameba histolytica*. In human cases, Anderson and his coworkers (6) were able to cure 33 of 39 and 41 of 44 cases treated with the dithioglycolate and dithiosalicylate, respectively, at total dosages which varied from seven grams given in a period of 24 hours, to three grams given over a period of 10 days. In human trypanosomiasis (*T. gambiense*) Friedheim (62) has reported initially favorable results with an "alkyl mercapto" derivative of melarsen oxide.

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TABLE 7

The relative toxicity and therapeutic activity of thioarsenites and the corresponding arsenoso compounds (39)*

—AsO = arsenosobenzene; —As(SR)$_2$ = corresponding dithioarsenite

	TREPONEMICIDAL ACTIVITY *in vitro*		TOXICITY IN WHITE MICE		THERAPEUTIC INDEX $\left(\frac{LD_{50}}{CD_{50}}\right)$			
					Mouse trypanosomiasis†		Rabbit syphilis	
	—AsO	—As(SR)$_2$	—AsO	—As(SR)$_2$	—AsO	—As(SR)$_2$	—AsO	—As(SR)$_2$‡
o-CH$_3$	84	109	88	90	—	—	—	—
p-OCH$_2$CONH$_2$	52	32	9.0	7.3	$\frac{33}{3.1} = 10.7$	$\frac{73}{7} = 10.4$	$\frac{10.5}{3.8} = 2.8$	$\frac{15\text{–}20}{4\text{–}6} = 3\text{–}4$
p-CONH$_2$	45	39 23a 21b	9.6	4.6 6.1 5.1	$\frac{27.5}{3.5} = 7.9$	$\frac{108}{6.9} = 15.7$	$\frac{9.1}{2.8} = 3.3$	—
p-NHCONH$_2$	38	21	8.1	2.9	$\frac{35}{3.8} = 9.2$	$\frac{180}{16} = 11.2$	$\frac{11.7}{4.6} = 2.5$	$\frac{35}{10} = 3.5$
p-CONHCH$_2$CONH$_2$	32	17	5.7	1.6	$\frac{79.5}{5} = 15.9$	$\frac{352}{16} = 22$	—	—
m-SO$_2$NH$_2$	21	22	6.1	3.3	—	—	—	—
p-SO$_2$NH$_2$	29	24	4.8	3.3	$\frac{63}{7.1} = 8.9$	$\frac{165}{21} = 7.9$	$\frac{16.1}{6} = 2.7$	$\frac{30\pm}{15\pm} = 2\pm$
3-NH$_2$-4-OH	38	29 0.86c	6.9	4.1 1.4	—	—	$\frac{13}{3} = 4.3$	$\frac{30}{8} = 3.8$
m-COOH	13	11	16	15	—	—	—	—
p-SO$_3$H	3.4	3.6	29	24	—	—	—	—

* All were dicysteinyl compounds, with the exception of the compounds indicated as a, b, and c, which were the dithiosalicylate, dithioglycolate, and dimercaptopropanol, respectively.

† *Trypanosoma equiperdum.*

‡ Approximations only.

TABLE 7

The relative toxicity and therapeutic activity of thioarsenites and the corresponding arsenoso compounds (39)*

—AsO = arsenosobenzene; $—As(SR)_2$ = corresponding dithioarsenite

	TREPONEMICIDAL ACTIVITY *in vitro*		TOXICITY IN WHITE MICE		THERAPEUTIC INDEX $\left(\frac{LD_{50}}{CD_{50}}\right)$			
					Mouse trypanosomiasis†		Rabbit syphilis	
	—AsO	$—As(SR)_2$	—AsO	$—As(SR)_2$	—AsO	$—As(SR)_2$	—AsO	$—As(SR)_2$‡
$o\text{-}CH_3$	84	109	88	90	—	—	—	—
$p\text{-}OCH_2CONH_2$	52	32	9.0	7.3	$\frac{33}{3.1} = 10.7$	$\frac{73}{7} = 10.4$	$\frac{10.5}{3.8} = 2.8$	$\frac{15\text{-}20}{4\text{-}6} = 3\text{-}4$
$p\text{-}CONH_2$	45	39 23a 21b	9.6	4.6 6.1 5.1	$\frac{27.5}{3.5} = 7.9$	$\frac{108}{6.9} = 15.7$	$\frac{9.1}{2.8} = 3.3$	—
$p\text{-}NHCONH_2$	38	21	8.1	2.9	$\frac{35}{3.8} = 9.2$	$\frac{180}{16} = 11.2$	$\frac{11.7}{4.6} = 2.5$	$\frac{35}{10} = 3.5$
$p\text{-}CONHCH_2CONH_2$	32	17	5.7	1.6	$\frac{79.5}{5} = 15.9$	$\frac{352}{16} = 22$	—	—
$m\text{-}SO_2NH_2$	21	22	6.1	3.3	—	—	—	—
$p\text{-}SO_2NH_2$	29	24	4.8	3.3	$\frac{63}{7.1} = 8.9$	$\frac{165}{21} = 7.9$	$\frac{16.1}{6} = 2.7$	$\frac{30\pm}{15\pm} = 2\pm$
$3\text{-}NH_2\text{-}4\text{-}OH$	38	29 0.86c	6.9	4.1 1.4	—	—	$\frac{13}{3} = 4.3$	$\frac{30}{8} = 3.8$
$m\text{-}COOH$	13	11	16	15	—	—	—	—
$p\text{-}SO_3H$	3.4	3.6	29	24	—	—	—	—

* All were dicysteinyl compounds, with the exception of the compounds indicated as a, b, and c, which were the dithiosalicylate, dithioglycolate, and dimercaptopropanol, respectively.

† *Trypanosoma equiperdum.*

‡ Approximations only.

the thioarsenite to the free arsenoso compound may be essential for parasiticidal activity. It is conceivable, however, that the thioarsenite reacts directly with a tissue thiol according to the equation

$$\begin{array}{ccccccc} \text{RAs(SR}')_2 & + & 2\text{R}''\text{SH} & \rightarrow & \text{RAs(SR}'')_2 & + & 2\text{R}'\text{SH} \\ \text{Thioarsenite} & & \text{Cellular} & & \text{Cell} & & \text{Free} \\ & & \text{thiol} & & \text{thioarsenite} & & \text{thiol} \end{array}$$

This reaction would involve the transfer of arsenic from one thiol directly to another. In such case an excess of thiol would inhibit parasiticidal activity, not by preventing hydrolysis but by competing with the cellular thiols for the thioarsenite (*cf.* page 130).

The finding of Peters and Stocken (91) that the condensation of "mapharsen" with BAL (2,3-dimercaptopropanol) results in a compound which in rats is four times more toxic than mapharsen itself has not been confirmed. A number of workers (63, 96, 100) have found that this thioarsenite also is approximately one fourth as toxic as the parent arseno compound. In mice the LD_{50} of the addition compound prepared in our own laboratory was 230 mg./kg. and 60 mM/kg. as compared with 43 mg./kg. and 12.4 mM/kg. for mapharsen.

B. The selective parasiticidal action of the arsenosobenzenes

The parent arsenosobenzene and those arsenoso compounds with "inert" substituents are highly toxic to the animal host and uniformly parasiticidal *in vitro* for all the organisms which have been tested. However, when substituents were introduced which modified the biological activity of the compound, they often developed a high degree of specificity. Thus, the amide-substituted compounds were relatively non-toxic for the mouse or rabbit host, uniformly active against *T. pallidum*, highly variable in their action on *Trypanosoma equiperdum*, and only negligibly active against *L. donovani* or *Trypanosoma cruzi* (Table 4). The acid-substituted compounds were generally only slightly parasiticidal. A few were highly active, however, against *Trypanosoma equiperdum*, *Trypanosoma cruzi* and *L. donovani*, and others were active against *T. pallidum* (*cf.* Table 3).

It is apparent that the relative activity of a series of compounds in the treatment of, *e.g.*, mouse trypanosomiasis cannot serve as a guide to their possible activity in the treatment of syphilis (93); nor do results obtained in the treatment of dog filariasis (*Dirofilaria immitis*) or cotton rat filariasis (*Litomosoides carinii*) necessarily apply to the treatment of the human infection with *Wuchereria bancrofti*. Similarly, results in the treatment of *Trypanosoma gambiense* infections in man do not necessarily carry over to the treatment of infections with *Trypanosoma rhodesiense* or *Trypanosoma cruzi*.

Possible reasons for this selective action are discussed in a following section.

C. Time–Dosage Relationship

The rate of the parasiticidal action of arsenosobenzenes *in vitro* increases with their concentration. Thus, in the immobilization of trypanosomes *in vitro*, a the thioarsenite to the free arsenoso compound may be essential for parasiticidal activity. It is conceivable, however, that the thioarsenite reacts directly with a tissue thiol according to the equation

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III. MODE OF ACTION OF ARSENOSO COMPOUNDS

A. The reactivity of arsenicals with thiols

Ehrlich originally suggested that drugs were effective only to the degree to which they were bound by "chemoreceptor" groups in the parasite, and laid down the thesis that "corpora non agunt nisi fixantur" (55). He further suggested that thiol groups might determine that fixation, although no definite chemical reaction was postulated (52).

The reaction between arsenosobenzenes and thiol compounds to form thioarsenites has been briefly discussed in a preceding section. In 1923 Voegtlin, Dyer and Leonard (120) demonstrated that trypanosomes contained —SH groups, that organic compounds such as cysteine and glutathione which contained —SH groups inhibited the trypanocidal action of trivalent arsenicals *in vitro*, and that the injection of such compounds immediately before an otherwise fatal dose of an arsenoso compound prolonged the life of the animal. They concluded that the effect of the arsenoso compounds *in vivo* was based on the reaction

$$RAsO + 2R'SH \rightarrow RAs(SR')_2 + H_2O,$$

and that the specific sulfhydryl compound which acted as the "chemoreceptor" in determining toxicity was primarily glutathione (121). The possibility that high concentration may have an instantaneous and total effect, while at threshold concentrations it may require four, eight or even 24 hours to immobilize only a fraction of the organisms. It is a reasonable assumption that the high concentrations are more rapidly effective, first, because the binding of the arsenical by the cell is correspondingly more rapid (*cf.* page 135), and second, because larger amounts are bound. These findings suggest that *in vivo* also, a single large dose might be essentially as effective as the same total dose given over a longer period of time in many divided injections. Fulton and Yorke (64) did find in *T. rhodesiense* infections that a low concentration of p-$NHCH_2CONH_2$ arsenosobenzene acting over a long period of time was as effective as a high concentration acting over a short period. In experimental trypanosomiasis (*T. equiperdum*) of rats, however, Swinyard and coworkers (112) found that the therapeutic efficacy of a series of small subcurative doses decreased the longer the time interval allowed between individual injections. In this case, the longer interval permitted rapid remultiplication of the surviving trypanosomes. In rabbit syphilis, Eagle and Hogan (43) found that the total curative dose of mapharsen (CD_{50}) was largely independent of the schedule of administration, whether the curative dose was given in one or 16 injections, or whether treatment was completed within one day or extended over six weeks. The disparity between these results and those of Swinyard is probably related to the fact that the generation time for *T. pallidum in vivo* has been estimated to be of the order of 30 hours (25, 81). An interval of even several days during which the arsenic was no longer at treponemicidal levels would therefore not permit remultiplication to an important degree.

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and that the specific sulfhydryl compound which acted as the "chemoreceptor" in determining toxicity was primarily glutathione (121). The possibility that other sulfhydryl-containing compounds might be involved was considered by a number of workers (97, 98, 104). The inhibiting effect of sulfhydryl compounds on arsenicals was extended to *T. pallidum* with the demonstration (33) that cysteine, glutathione and thioglycolic acid in excess abolished the treponemicidal action of arsenicals *in vitro*, as well as that of mercury and bismuth compounds. *In vivo*, thiols and dithiols in appropriate dosage decreased or abolished both the toxic and parasiticidal effects of arsenoso compounds even when administered hours after otherwise lethal (or curative) doses ((38, 50, 92); *cf*. Fig. 5).

The antagonistic effect of thiols on arsenosobenzenes as outlined in the foregoing paragraphs involves two quite different mechanisms. Thiol compounds in excess react with arsenicals to form thioarsenites, inhibit the hydrolysis of the formed thioarsenites to the free arsenoso compounds, and thus prevent the interaction of the latter with the host tissues or with the parasites. In addition, however, even after arsenosobenzenes have combined with the tissue cells or parasites, thiol compounds may reverse that combination, and remove the arsenical from its combination with the cells (34, 38, 50). When cysteine was added to a suspension of *Treponema pallidum* which had previously been incubated with arsenicals and in which the larger proportion of the organisms had already been immobilized, not only was the treponemicidal action of the arsenical immediately halted but a large proportion of the already immobilized organisms were revived (34). The same phenomenon has been even more strikingly demonstrated with trypanosomes "killed" by arsenical and then exposed to BAL (2,3-dithiopropanol) (50). *In vivo* also, it is apparent from Fig. 5 that the parasiticidal action of arsenoso compounds may be successfully reversed by

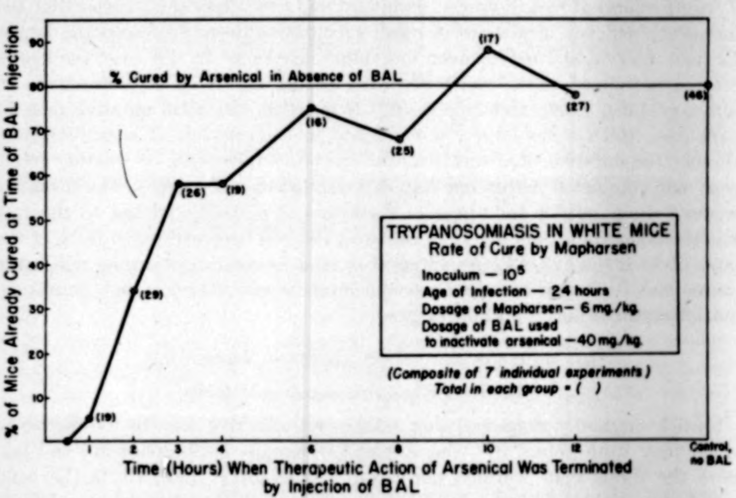


FIG. 5. The inhibition of the trypanocidal action of oxophenarsine in white mice by 2,3-dithiopropanol (BAL). (After 38.)

thiol compounds given hours after the arsenical had been injected, and after the organisms had been given ample opportunity to combine with the injected arsenical. Similar results have been reported in rats by Pfeiffer, Jenney and Ross (92). In experimental syphilis also, BAL given up to six hours after an other wise curative dose of oxophenarsine reversed the therapeutic action in a significant proportion of the animals (39). The same compound decreased the lethal action of a number of arsenoso compounds in rabbits (50). The ability of thiol compounds to abstract arsenicals from the cell after they have already entered into combination with cell components is manifested not only in the revival of microorganisms or animals already seriously damaged by the arsenicals, but may be demonstrated by the direct chemical analysis of the cells affected (50). The arsenical presumably leaves the cell in the form of the thioarsenite. In the animal host also, certain thiols have been shown to counteract strikingly the toxic effects of arsenosobenzenes, and that effect likewise is associated with the removal of arsenic from the host cells and with its accelerated excretion (*cf.* page 128).

B. *The reactivity of arsenicals with —SH groups in enzymes*

The fact that arsenicals react strongly with thiol compounds to form thioarsenites, and also the fact that thiol compounds are able to remove arsenic after it has already entered into combination with cellular constituents do not, however, prove that a similar combination with cell thiols is responsible for the toxic action of arsenicals. More recent work has indicated that this may actually be the case, and that the reactivity of arsenicals with —SH groups in protoplasm is the factor which determines their cytotoxic effects. It now seems unlikely that glutathione as such is necessarily or even primarily the cellular grouping affected.

In 1933 several workers reported on the role of thiol groups in the reversible deactivation of enzymes. Hellerman, Perkins and Clark (72) studied the inactivation of crystalline urease with mercurials such as phenylmercuric hydroxide, and Bersin and Logermann (14) noted that the activity of papain was destroyed by many oxidizing agents. In both instances, the enzymatic activity was restored by the addition of either hydrogen sulfide or potassium cyanide. Maschmann (83) had suggested that such reactivation of papain or cathepsin by H_2S involved reductions of disulfide linkages in the enzyme to active thiol groupings essential for activity.

Bersin (13) then correlated the work of Cohen, King and Strangeways (21) on the reaction between arsenicals and thiol compounds *in vitro* and the several previous indications as to the importance of thiol groups in the activity of many enzymes, and systemically studied the effects of a number of arsenic compounds on papain. He showed that papain was inhibited by both arsenoso compounds and arsonic acids, and that this inhibition was reversed by glutathione. In explanation, he proposed the following alternative reactions:

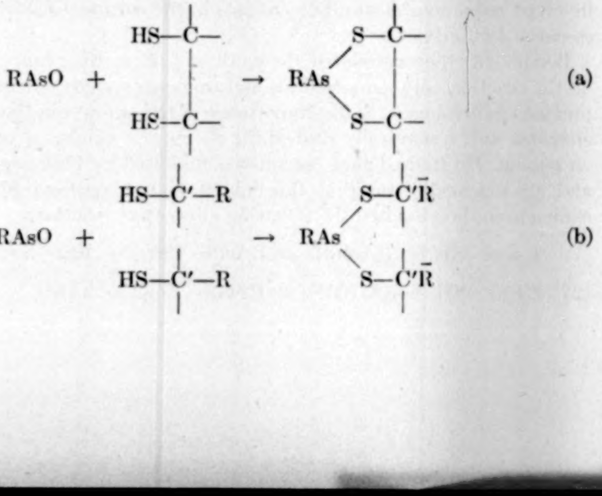
(1) $4\ \text{Enz—SH} + \text{RAsO}_3\text{H}_2 \rightleftarrows \text{RAs(S—Enz)}_2 + \text{Enz—S—S—Enz} + 3\text{H}_2\text{O}$

(2) $2\ \text{Enz—SH} + \text{RAs(OH)}_2 \rightleftarrows \text{RAs(S—Enz)}_2 + 2\ \text{H}_2\text{O}$

A marked difference in the inhibitory actions of the p-acetamido- and p-aminophenylarsonic acids was explained on the basis of a difference in the equilibrium constant in reaction (1) above.

Barron and his coworkers (9–12) have studied the inhibiting effect of arsenicals on a number of enzyme systems, including enzymes involved in nitrogen, fat and carbohydrate metabolism. Many were reversibly inhibited by arsenoso compounds and by the war gas "Lewisite" ($ClCH = CH\ As\ Cl_2$); in such cases, —SH groups in the enzyme protein were believed to be essential to their enzymatic activity. Similarly, Gordon and Quastel (67, 68) found that arsenoso compounds reversibly combined with and inactivated such thiol enzymes as urease, pyruvic oxidase, succinic dehydrogenase and choline esterase, while enzymes in which —SH groups were presumably not part of the active centers, such as invertase, catalase, lactic dehydrogenase and cytochrome oxidase, were unaffected. The inactivating action of the arsenicals on the susceptible enzymes was reduced or prevented by the addition of free thiols or of other proteins capable of combining with the arsenical. Chen (19a) also found mapharsen to inhibit hexokinase, adenosine triphosphatase and 3-phosphoglyceraldehyde in preparations of lysed *T. equiperdum*. Conversely, dithiols have been found to inhibit certain enzymes in which the protein moiety has a heavy metal prosthetic group (10, 123).

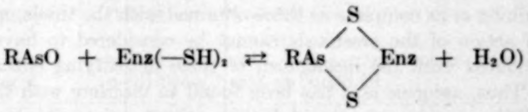
In England, Peters and his coworkers (90, 91, 107–110, 124) have extensively investigated the reaction of enzymes and trivalent arsenicals. They considered the primary site of attack of the arsenicals in the animal body to be on the pyruvate-oxidase system, which is particularly sensitive to trivalent arsenicals. Stocken and Thompson (107) reacted sodium arsenite and Lewisite with kerateine in buffered solution and showed that from 73 to 92 per cent of the bound arsenic had combined with protein thiol groups in the molecular ratio 1:2. Further, the protein sulfur was now resistant to oxidation, while the thiol groups in kerateine are normally highly susceptible. They therefore postulated that the arsenic had combined with two neighboring thiol groups on the protein molecule, *i.e.*, that the protein contains a dithiol which is the primary reactive site for arsenicals. On physicochemical grounds the ring compound formed by the reaction of a dithiol with an arsenical (a) was believed to be more stable than the straight chain thioarsenite formed with 2 molecules of a monothiol (b):



There may be several gaps in this argument. No dithiol such as that postulated by Stocken and Peters has yet been identified as a constituent of kerateine or any other protein in the amounts implied by their experimental data. It seems unlikely also that arsenicals would react with —S—S— groups in proteins, or that the major portion of the free monothiol groups in a protein molecule would be arranged in closely adjacent pairs. The fact that the arsenic: sulfur ratio of the compound formed between thiols and kerateine in solution approaches 1:2 is not conclusive evidence for the presence of a dithiol, since the arsenic molecule could be bridging two molecules of kerateine. Further, in the absence of resonance there seems to be no necessary basis for assuming that a bond between two atoms in a ring is necessarily stronger than the same bond in a straight chain compound. Whittaker (124) did find that those dithiols which he believed to react with arsenic to produce strained rings were less effective in counteracting the toxic effects of arsenic than dithiols which formed a stable ring structure. This does not, however, prove that the latter are necessarily more stable than a straight chain compound. Slater (106) has furnished strong evidence that the reaction between arsenicals and enzyme proteins may be more complex than that suggested by Peters and his coworkers. He has pointed out that while the reaction is supposed to be:

$$\mathrm{RAsO} + 2\mathrm{HS{-}Enz} \rightleftarrows \mathrm{RAs(S{-}Enz)_2} + \mathrm{H_2O}$$

(or, on the basis of the Stocken-Peters concept,



there may be no reactivation of the enzyme when the arsenical is removed or the reaction mixture is greatly diluted.

However, whether the reasoning of Stocken and Peters is valid in its entirety, it has led to a significant advance in the treatment of heavy-metal poisoning. Following up the previously cited data, Stocken and Thompson (108) found that dithiols, and particularly BAL (British Anti-Lewisite; 2,3-dimercaptopropanol; $\mathrm{HSCH_2CH(SH){-}CH_2OH}$) were more effective than monothiols in protecting the pyruvate-oxidase system in pigeon brain against arsenicals. In animals suffering Lewisite burns or injected with toxic arsenicals, the dithiols greatly increased the rate of excretion of arsenic (18, 49, 96, 107–110) and strikingly counteracted the toxic effects of a number of trivalent arsenicals in experimental animals. As an outcome of these studies, BAL has found extensive use in the local and systemic treatment of arsenic poisoning (18, 49, 50, 78) and poisoning due to such heavy metals as mercury (79), antimony (16, 42) and gold (20, 94). It is ineffectual against cadmium poisoning (66) and of questionable value in lead poisoning (65, 99).

Summarizing the above evidence, one may conclude that (a) arsenoso compounds combine with sulfhydryl compounds to form reasonably stable thioarsenites; (b) many enzyme groups contain sulfhydryl groups which are necessary in the intact state for the action of the enzyme, and such enzymes are inactivated by arsenoso compounds and other heavy metal compounds capable of reacting with —SH groups; (c) this inactivation can be reversed by thiols, and in particular by certain dithiols; (d) thiol compounds, and particularly dithiols, protect animals and microorganisms against the toxic effects of arsenicals, can on occasion reverse the toxic action of arsenicals after they have already become manifest, and in such cases actually reverse the combination of the arsenical with the cell or tissue.

On the basis of these facts it is a generally-accepted working hypothesis that the cytotoxic action of the arsenosobenzenes rests on their ability to combine with thiol groups in essential enzyme systems, both in the animal host tissue (toxicity) and in the invading parasitic cell (therapeutic activity). This may be expressed schematically as

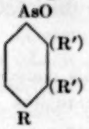
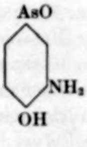
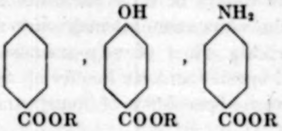
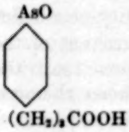
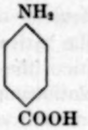
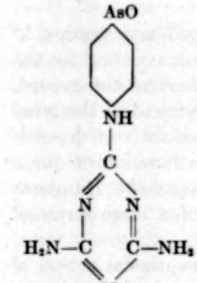
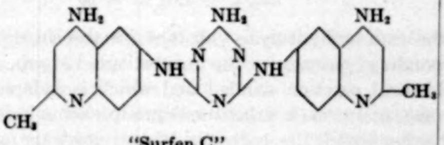
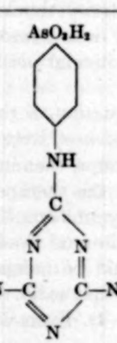
$$\underset{\substack{\text{arsenoso}\\\text{compound}}}{\mathrm{RAsO}} + \underset{\substack{\text{enzyme in}\\\text{host tissue}\\\text{or in para-}\\\text{sitic cell}}}{2\mathrm{R'SH}} \rightleftarrows \underset{\substack{\text{tissue or cell}\\\text{thioarsenite}}}{\mathrm{RAs(SR')_2}} + \mathrm{H_2O}$$

C. Other arsenic-detoxifying mechanisms

A number of compounds other than thiols have been shown to prevent or to reverse the cytotoxic action of arsenicals in varying degree. In no case are the results as striking or as complete as those obtained with the thiols; nevertheless, the mode of action of the arsenicals cannot be considered to have been adequately elucidated until the mechanism of these detoxifying effects has been established. Thus, ascorbic acid has been found to interfere with the action of mapharsen (58); a number of quinoid dyes constituting reversible redox systems in a narrow potential range similarly interfered with the trypanocidal effect of 3-NH_2-4-OH arsenosobenzene (74); para-aminobenzoic acid was found to interfere with the trypanocidal action of "butarsen" (para-arsenosophenylbutyric acid) (125) but not that of melarsen oxide or "mapharsen" (126) on *T. rhodesiense*. The trypanocidal action of the latter compound was, however, antagonized by a number of benzoic acid esters and amides (103). Finally, Surfen C (bis-(2-methyl-)4-amino-6-quinolyl-melamine) interfered selectively with the action of "melarsen oxide," as did also the pentavalent analog of the latter compound, "melarsen" (126). There is no obvious common denominator for the antagonistic action of these widely disparate compounds on arsenosobenzenes (Table 8). Further, these inhibitory effects are not necessarily associated with an inhibition of toxicity (103). In several cases the antagonistic effect may conceivably be related to a similarity in chemical structure. It seems unlikely that these inhibitors would compete with the arsenoso compound for SH groups in an enzyme protein. The latter reaction is determined by the —AsO grouping, and there is no evidence that compounds which are sterically similar but which lack the —AsO group would have a similar reactivity. Williamson and Lourie

TABLE 8

The inhibition of the cytotoxic action of arsenosobenzenes by a variety of agents

ARSENICAL TESTED	PARASITICIDAL ACTION INHIBITED BY:	TEST ORGANISM	REFERENCES
Arsenosobenzenes in general 	**Thiols generally (—RSH)**	***T. pallidum; Tryp. equiperdum,* etc.**	**(*cf.* pages (125–127))**
 **Oxophenarsine ("mapharsen")**	**Quinoid dyes** **Ascorbic acid**  **and corresponding substituted amides (—CONHR) (but *not* by p-aminobenzoic acid)**	***Tryp. brucei***	**74** **58** **103**
 **γ-p-Arsenosophenylbutyric acid**	 **p-aminobenzoic acid (but *not* by benzoic acid, p-OH-benzoic acid, or Surfen C)**		**125**
 **"Melarsen oxide"**	 **"Surfen C"**  **"Melarsen" (but *not* by p-aminobenzoic acid)**	***T. rhodesiense***	**126**

(126) have suggested that the site of the competitive inhibition is not the specific cell component inactivated by the arsenical, but perhaps a surface configuration on the cell which constitutes the portal of entry for the drug (*cf.* page 137).

D. Possible explanations for the varying reactivity of different arsenicals against the same organism, and of the same arsenical against different organisms

How does the theory of reactivity of arsenicals with enzyme —SH groups explain the experimental observations, cited in the preceding sections, 1) that a series of chemically closely related arsenoso compounds may vary 100-fold in toxicity, and vary just as widely in their parasiticidal action on a given microorganism, and 2) that the same arsenical may vary widely in its effect on different organisms? The striking effect of γ-(p-arsenosophenyl)-butyric acid against certain trypanosomal species and its inactivity against *T. pallidum* is but one example of the parasite-specificity of many arsenic compounds (*cf.* Tables 1–4).

1. *The possibly varying reactivity of different arsenicals and thiols as the basis of their parasite specificity.* The reaction indicated on page 125 is freely reversible, being shifted to the left in alkaline solution and to the right in acid solution (21). It is therefore conceivable that the variation in toxicity or activity of a series of arsenoso compounds might be a function of the hydrolysis constant of the thioarsenite formed. It is also possible that the rate of the above reaction is the determining factor. In either case, the usual relationships between chemical structure and reactivity should apply. In this particular case it should be possible to predict both the relative rate of the reaction and the hydrolysis constant by means of Hammett's equation (70):

$$\text{Log k} - \text{Log k}^0 = \rho\sigma$$

where k is the rate or hydrolysis constant for the substituted aryl groups, k^0 is the corresponding constant for the unsubstituted group, ρ is a constant for the particular chemical reaction studied and which is independent of the groups, and σ is a constant for each substituent group and is independent of the reaction (70). If either toxicity or parasiticidal activity were quantitatively dependent closely on the reaction above, that activity should be a function of the σ values. Actually, no correlation whatever was found between the Hammett σ values and either the toxicity or parasiticidal activity of a large series of arsenoso compounds (27).

Again on the basis of the Hammett equation, if the parasiticidal action of arsenicals and their toxicity to the animal host were both quantitatively dependent upon their reactivity with thiol groups, then in a given infection involving the same parasite and the same host, the therapeutic index of a series of arsenoso compounds should be reasonably uniform. The reactivity of an arsenoso compound with thiols in the parasite and thiols in the host cell should vary concomitantly, and their ratio should be independent of the particular substituent on the benzene ring. Instead, that ratio varied as much as 60-fold in a relatively limited series (*cf.* Tables 1–4). Even when the same compound was tested against the same organism (*Trypanosoma rhodesiense*) but in different animal hosts, Tatum, Pfeiffer and Kuhs (115) obtained therapeutic indices of 20–25, 4–5 and 5 in rats, rabbits and dogs, respectively.

It seems clear that possible differences in the rate or equilibrium constants of the reaction between arsenoso compounds and thiols to form thioarsenites are inadequate to explain the known variations in the parasiticidal action or toxicity of these compounds.

The studies of Barron and Singer (11, 12) on the relative inhibitory action of various arsenicals against a number of enzymes *in vitro* are pertinent in this connection, although the quantitative aspects of their data are partially obscured by the varying amounts of tissue impurities in the enzyme preparations which were capable of combining with and inactivating the arsenicals. The susceptibility of the different enzymes to the same arsenical was generally of the same order of magnitude (11). Using eighteen different enzyme systems, they found that under the particular experimental conditions, most of the enzymes were almost completely inhibited (by 80 to 100 per cent), but that pyruvate dismutation was inhibited by only 35 per cent and pyruvate condensation by 56 per cent. The degree of inhibition obtained with five different arsenoso compounds acting on the same enzyme, succinoxidase, were also not significantly different (12). These findings suggest that factors may supervene in the reaction of arsenicals with formed cells which do not complicate their reactivity with enzyme proteins in solution. However, from the same laboratory it was later shown that *d*-amino acid oxidase and yeast carboxylase were inhibited by p-aminophenyldichlorarsine but not by Lewisite (7). Also, transaminase was almost completely inhibited by p-arsenosobenzoic acid, but only 33 per cent inhibited by the same concentration of Lewisite. The authors suggest that these differences may arise from a different spatial arrangement of the thiol groups on the enzyme protein.

2. *The possibly varying permeability of the cell and a consideration of "arsenic-resistance."* In attempting to explain the widely varying biological activity of arsenosobenzenes and their selective parasiticidal action, consideration must be given the fact that the unsubstituted arsenosobenzene is highly toxic for all cells against which it has been tested, and that substituent groups generally depress that activity. The specificity of arsenosobenzenes is thus not an activity newly bestowed upon the compound by the substituent group, but results rather from the fact that the activity against one cell type has been suppressed to a lesser extent than that against other cells. It follows that the reactive grouping is the arsenoso (—AsO) grouping, common to all the arsenosobenzenes, and not the other substituents. How the latter serve to modify the reactivity of the —AsO grouping is a moot question. No evidence has yet been presented that substituent groups affect either the dissociation constant of the —AsO group or its reactivity with SH compounds. There is no evidence that differently substituted compounds react to a widely varying degree or at widely varying rates with a given SH compound, or that the same arsenosobenzene will react differently with different SH compounds.

As an alternative explanation of the varying biological effects of different arsenoso compounds, one may consider the possibility that although their reactivity with SH compounds is reasonably uniform, the arsenicals vary widely in their ability to penetrate the cell wall, either in the microorganism or the host, and that it is these differences which largely determine the varying parasiticidal action and toxicity of these compounds. This thesis is strongly supported by the data on the binding of arsenicals by cell suspensions. It was first shown by Thuret (117) that a suspension of red blood cells *in vitro* bound the highly active arsenoso compounds, but not the relatively inactive arsonic acids. This finding was extended by Hogan and Eagle (73), who demonstrated that in a large series of arsenoso compounds the amount of arsenic bound by red blood cells *in vitro* under standard conditions was roughly proportional to the systemic toxicity of the arsenical, strongly suggesting a causal relationship (Fig. 6). The corollary of this finding was also demonstrated: when compounds of widely varying toxicity were injected at dosages which produced equivalent toxic effects *in vivo*, essentially similar amounts of arsenic were bound by the tissues of the host.

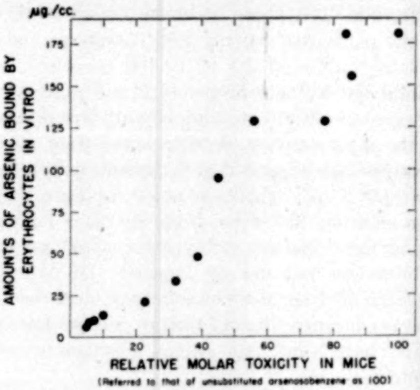


FIG. 6. The correlation between the affinity of arsenosobenzenes for erythrocytes *in vitro* and their systemic toxicity in mice. (After 61.)

(The binding of arsenicals by erythrocytes was also studied by Fink and Wright (57). With oxophenarsine, these workers found an approximate linear relationship between the logarithm of the amount in the cells and the logarithm of the amount in the plasma, and found also that from 60 to 90 per cent of the bound arsenic could be released from the red blood cells by simple resuspension in fresh plasma. They therefore suggested that the binding of arsenicals was largely physical in nature. These important findings are difficult to reconcile with the thesis that arsenoso compounds react with —SH groups in the cells as previously discussed, and further study seems indicated.)

Eagle and Magnuson (48) subsequently showed that, just as the degree to which arsenicals were bound by red blood cells was related to the systemic toxicity of the compounds, so the binding of arsenicals by trypanosomes was a measure of their parasiticidal activity (Table 9). The correlation between the amount of the arsenical bound under standard conditions and its trypanocidal activity was again so regular as to suggest a causal relationship. This quantitative study confirmed and extended the previous qualitative findings of Yorke, Murgatroyd and Hawking (130), Hawking (71) and Reiner, Leonard and Chao (95) that actively trypanocidal compounds were bound by the organisms, while inactive compounds were not.

The wide differences in the amounts of arsenical bound by a trypanosomal or erythrocyte suspension could perhaps be due to corresponding differences in the reactivity of the several arsenicals with a given cellular enzyme system. Differences of this order of magnitude have, however, not been demonstrated in the reaction of arsenoso compounds with thiols *in vitro;* it seems unlikely that the hydrolysis constant of a series of thioarsenites involving the same thiol and different arsenoso compounds would differ by a factor of, *e.g.*, 10^2–10^4 (*cf.* pages 111–120). However, the results are consistent with the thesis that different arsenicals vary in the ease with which they can pass through the cell wall, and that it is this selective permeability which determines the varying activity of a series of closely related compounds against the same cell.

TABLE 9

The relationship between the trypanocidal activity of arsenosobenzenes and their combining affinity with the organisms. (After 48)

Original As concentration = 0.166 mg. %; no. of trypanosomes = 250×10^6 cc.; vol. of 10^9 sedimented organisms = 0.48 cc.

SUBSTITUTED ARSENOSOBENZENE R—AsO	RELATIVE TRYPANOCIDAL ACTIVITY (REFERRED TO ARSENOSOBENZENE AS 100)	CONCN. OF As IN TRYPANOSOMES MG. %	AVERAGE RATIO OF $\frac{[As]\ \text{TRYPANOSOMES}}{[As]\ \text{SUPERNATANT}}$
p-SO_3H	0.06	0.41	2.4
p-$SO_2NHCH_2CONH_2$	1.4	0.68	5.2
p-OCH_2COOH	4.5	1.5	11
p-$CONHCH_2CONH_2$	15	5.7	53
3-NH_2-4-OH	27	6.5	87
p-$SO_2N(C_2H_5)_2$	35	7.4	93
p-$CONH_2$	45	7.7	113
m-OH	66	8.2	119
o-CH_3	91	9.5	186
Unsubstituted arsenosobenzene	100	9.7	224

The results obtained with "arsenic-resistant" strains of trypanosomes offer additional strong evidence that the varying amounts of arsenical bound by the organisms reflect the varying permeability of the cell wall to the several compounds, rather than their varying reactivity with a cell thiol or thiols. Hawking (71) showed that unsubstituted arsenosobenzene was as actively trypanocidal against an "arsenic-resistant" strain of *T. equiperdum* as against a normal strain; but that arsenoso compounds containing certain substituents, notably the $—NHCH_2CONH_2$ group, were not effective against the resistant organism and were not bound by it. Similar results were obtained by Yorke, Murgatroyd and Hawking (130), using an arsenic-resistant strain of *T. rhodesiense*. On the basis of their studies with an arsenic-resistant strain of *T. rhodesiense*, King and Strangeways (75, 76) concluded that arsenicals may act on these organisms in at least three different ways. Arsenosobenzene, arsenosoxylene, and similar compounds which fall into the group of compounds with "inert" substituents as defined on page 111 were equally active against both the normal and "arsenic-resistant" strains. The resistant organisms were resistant to several amide- and acetamido-substituted compounds which were highly active against the normal strain, while several acid-substituted compounds had an equally low measure of activity against both the normal and resistant variants. They concluded that while the actual intracellular process which determines the death of the organism is the same in each instance, the several types of compound entered the trypanosome by different mechanisms. Eagle and Magnuson (48), working with a strain of *T. equiperdum* which had spontaneously become "arsenic-resistant," found that arsenosobenzene, many of its simple derivatives (chloro-, methyl-, and nitro-, etc.), and arsenosobenzenes with substituents carrying a terminal acidic group were just as active against this resistant variant as against the parent normal strain and were bound just as strongly by it. On the other hand, arsenoso compounds containing terminal amide, amine or acetamide substituents were $\frac{1}{5}$th to $\frac{1}{200}$th as active against the resistant strain as against the normal strain, and the amount of arsenical bound was correspondingly reduced. In human trypanosomiasis, "arsenic-fast" strains of *T. gambiense* which pose so serious a therapeutic problem in large areas of Central Africa have regularly proved resistant to amide-substituted arsenicals (*e.g.*, tryparsamide), but are normally susceptible to γ-(p-arsenosophenyl)-butyric acid and to melarsen oxide (37, 61, 119).

It is thus well established that so-called "arsenic-resistant" trypanosomes are normally susceptible to certain types of arsenicals and that this susceptibility is associated with the ability of these arsenicals to combine with the cell. The only simple explanation is a selective permeability of the cell for certain arsenicals. Otherwise, one must assume that arsenicals with contrasting effects on "resistant" strains (*e.g.*, acid- and amide-substituted compounds) combine selectively only with certain thiols in the cell and, further, that normal and resistant trypanosomes differ qualitatively with respect to their thiol content. The former assumption in particular seems improbable.

The importance of cell permeability is further indicated by the fact that, with acid substituted arsenoso compounds, the activity against both *Tryp. equiperdum* and *T. pallidum* decreased strikingly as the pH of the testing solution was increased (*cf.* page 116), and the degree to which the compounds were bound by the organisms at varying pH decreased concomitantly. It thus appears that the ion is relatively inactive because it is bound by the organism to only a limited degree, while the undissociated acid is highly active because it is strongly bound. These differences can hardly be related to the varying reactivity of the salt and of the free acid with thiols; the salts of acid-substituted arsenoso compounds react as readily with cysteine and other thiols as do arsenoso compounds with non-ionic substituents. However, the results are consistent with the thesis that the ionized form passes into the interior of the cell only to a limited degree as compared with the undissociated molecule. The many analogies with other types of compounds and other cell species have been summarized by a number of workers (1, 36, 105). (The possibility that the arsenicals are bound on the outside wall of the organism, rather than within the cell, has been discussed by Reiner, Leonard and Chao (95) and by Eagle (36). The latter concluded that the quantitative relationships between the amount of arsenical bound and the available surface area make it unlikely that the major portion of the arsenic bound by trypanosomes attaches to the cell membrane.)

According to the permeability theory, the cell wall would be relatively impermeable to certain arsenoso compounds whereas others can penetrate with ease. Having penetrated, the arsenicals are so firmly bound to some of the cellular constituents (thiols) that they no longer figure in the intracellular-extracellular equilibrium. In consequence, the drug continues to diffuse into the cell, and the total intracellular concentration continues to build up, attaining 50, 100 and even 200 times that in the surrounding fluid. The factors determining which compounds can penetrate the cell wall and which cannot are not clear. Unresolved also is the nature of the changes in the "resistant" cell which make it almost wholly impermeable to some compounds which are normally highly active.

A number of drugs have been found to antagonize the cytotoxic effects of arsenicals (*cf.* page 130). Unfortunately, there are no data as to whether these antagonists actually prevent the concentration of the arsenical within the cell. If that proves to be the case, the antagonists may either prevent the passage of the arsenical across the cell wall or, by blocking the union of the arsenical with an intracellular compound, prevent its rapid accumulation and parasiticidal action. Work and Work (127), commenting on the striking antagonism of p-aminobenzoic acid for γ-(p-arsenosophenyl)-butyric acid, concluded that "the only explanation for this action is to assume that the drug acts ultimately in the same manner as other trypanocidal arsenicals, but that PABA may be preventing or limiting its admission into the trypanosome cell."

IV. SUMMARY

1. Of the three types of arsenicals used in the treatment of spirochetal and protozoal diseases, only the arsenoso compounds have a direct parasiticidal activity, the arsonic acids and arseno compounds being active by virtue of their conversion to arsenoso compounds in the animal host.

2. Unsubstituted arsenosobenzene was one of the most toxic and most actively parasiticidal compounds in the entire series tested, with no demonstrable selective action among the organisms studied.

a) Substitution with a single —CH_3, —NO_2, —Cl, —NH_2, —OH, or —F group either did not significantly affect the toxicity or parasiticidal activity of the parent arsenosobenzene, or reduced them both to the same slight degree.

b) Acid-substituents strikingly decreased both the direct parasiticidal and toxic action of arsenosobenzene. The ionized form was generally inactive, the undissociated acid highly active, and the effect of pH on parasiticidal activity could be related to its effect on dissociation. However, there were important exceptions to the general inactivity of the ionized compounds (*e.g.*, the 3-NO_2-4-COOH and p-$(CH_2)_3COOH$ arsenosobenzenes); several compounds in this series are of distinct therapeutic interest.

c) Amide substituents usually caused a slight decrease in treponemicidal and trypanocidal activity, but a striking decrease in toxicity. As a class, they are therapeutically the most promising group of arsenoso compounds so far studied. Substitution in the amide hydrogens usually diminished its favorable effect on toxicity, exceptions being noted in groups which also reduced the toxicity of arsenosobenzene when substituted directly onto the benzene ring (*e.g.*, $—C_2H_4OH$, $—C_6H_4NHCOCH_3$).

d) Unlike amidification, esterification of acid substituents resulted in compounds approaching arsenosobenzene in their high toxicity and general parasiticidal action. Many of these compounds readily hydrolyzed in aqueous solution, their biological activity then corresponding to that of the free acid.

e) The effect of complex substituents was usually determined by the nature of the terminal group in the substituent (*e.g.*, $(CH_2)_3CONH_2$; $CON(CH_3)_2$).

f) Two substituent groups had an effect which could not be predicted from those of the substituents taken singly. Unlike the case of the single substituents, in these di-substituted compounds the position on the benzene ring profoundly modified the activity of the compound.

g) Both the toxicity and direct parasiticidal activity of thioarsenites were somewhat less than those of the corresponding arsenosobenzene; in the treatment of rabbit syphilis and mouse trypanosomiasis, the therapeutic index was no better than that of the parent compound.

3. There are several instances of arsenoso compounds with a therapeutic index in a particular infection greatly exceeding that of chemically closely related compounds (*e.g.*, "mapharsen," "melarsen oxide," p-arsenosophenylbutyric acid). These exceptions emphasize the unreliability of generalizations with respect to the effect of a given type of substituent on the biological activity of arsenosobenzene.

4. There is considerable evidence to support the view that the toxicity and therapeutic activity of arsenoso compounds are determined by the amounts which enter into combination with cellular components. The widely varying activity of a single arsenoso compound against different organisms, and of a series of such compounds against the same organism, is related to the amount of arsenical bound.

5. It is a reasonable working hypothesis that arsenoso compounds combine reversibly with sulfhydryl groups in essential enzyme proteins, and that their cytotoxic effect is due to that combination. Many essential enzymes contain sulfhydryl groups, and most such enzymes can be inactivated by arsenoso compounds and reactivated by sulfhydryl or other agents which can compete with the enzyme protein for the arsenical.

6. The highly selective action of particular arsenoso compounds against certain cells could be explained on the basis of either (a) the varying affinity of different enzymes for the same arsenical, or of different arsenicals for the same enzyme, or (b) differences in the permeability of a given cell to different arsenicals. While the evidence is not conclusive, the latter assumption appears best to explain the available data.

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METHEMOGLOBINEMIA AND METHEMOGLOBIN-PRODUCING COMPOUNDS

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I. INTRODUCTION

A great variety of drugs are known to convert hemoglobin into methemoglobin and thereby to impair the blood's capacity to transport oxygen to the tissues. In addition, a number of substances which are used in industrial processes possess this property. Nitrates in food and water which are of themselves innocuous may, by the action of intestinal bacteria, be converted into nitrites and so form methemoglobin. Finally, there are a number of instances, fortunately rare in man, in which, without obvious external cause, methemoglobin is formed from hemoglobin and constitutes a constant hazard to the well-being of the individual.

Without attempting any exhaustive tabulation of the vast literature, the present review is directed towards correlating available information concerning the chemistry of methemoglobin, the biochemical relationships between hemoglobin and methemoglobin, the extent and nature of the physiological impairments produced by methemoglobinemia, the description of the substances which form methemoglobin and the mechanisms by which they do so, and finally the principles and methods to be followed in the treatment of methemoglobinemia.

II. CHEMISTRY OF METHEMOGLOBIN

A. *Structure*

Methemoglobin is an oxidation product of the normal blood pigment, hemoglobin. The prosthetic group of hemoglobin is variously known as heme, ferroheme (Pauling and Coryell (193); Barron (16)), or ferroporphyrin (Clark (45); Drabkin (65)) and is a complex of iron and protoporphyrin IX, a type III porphyrin. The essential structure of the porphyrins is a nucleus of four pyrrole groups united by four methene, =CH—, linkages. Protoporphyrin IX is 1,3,5,8-tetramethyl-2,4-divinylporphin-6,7-dipropionic acid. The iron in this structure may be considered as coordinated with the four nitrogen atoms, thus leaving two more coordination bonds available. In methemoglobin, iron is present in the ferric state. Methemoglobin is also known as hemiglobin and ferrihemoglobin.

The nature of the linkage between the globin and the prosthetic group in hemoglobin or methemoglobin has received considerable attention. Though there is much evidence that this combination occurs through a linkage of the iron with the imidazole groups in the globin molecule, the carbamino reaction of hemoglobin does not appear to fit in with such a hypothesis. Clark (47) considers that the exact nature of the bonding between the prosthetic group and the specific protein, globin, is still unknown. This field has been admirably reviewed by Lemberg and Legge (153).

B. Formation of methemoglobin

Methemoglobin may be formed from hemoglobin in a variety of ways. Quite obviously, the essential action is an oxidation of the ferrous to the ferric ion, but such oxidation may be brought about in one of several ways: (a) by the direct action of oxidants; (b) the action of hydrogen donors in the presence of atmospheric oxygen, and (c) autoxidation. In addition, many of the substances which are used to effect this oxidation combine with hemoglobin or methemoglobin.

The ferrous ion of hemoglobin may be oxidized directly by ferric tartrate, ferricyanide, bivalent copper, chlorate, nitrate, quinones, alloxans and dyes of high oxidation-reduction potential (33, 62, 153, 175, 206, 213).

Another mode of oxidation is that of the action of hydrogen donors in the presence of atmospheric oxygen. Chief representatives in this group are the dyes (62, 177). Some of these have oxidation-reduction potentials which are greater than that of methemoglobin, the E_0 of which is 150 millivolts. Such, for example, are phenolindophenol with a potential, E_0, of 250 millivolts. However, a number of the most important methemoglobin-forming dyes such as methylene blue with an E_0 of 10 millivolts have oxidation-reduction potentials below that of methemoglobin.

The oxidizing action of methylene blue may be explained in either of two ways. First, although the E_0 value of methemoglobin is greater than that of methylene blue, the latter is conceived of as interacting with a small fraction of the hemoglobin to form methemoglobin and leucomethylene blue. The latter is then reoxidized to methylene blue by atmospheric oxygen, and a cyclic reaction is set up whereby all the hemoglobin is finally oxidized to methemoglobin. The second explanation is that of Lemberg and his coworkers (153, 154) who have postulated that a hydrogen donor such as leucomethylene blue may undergo autoxidation with the formation of hydrogen peroxide which either oxidizes hemoglobin directly to methemoglobin or else forms an intermediate with oxyhemoglobin; the latter then decomposes to yield methemoglobin:

$$\mathrm{HbO_2 + H_2A \rightarrow Hb\cdot H_2O_2 + A}$$
$$\mathrm{Hb\cdot H_2O_2 \rightarrow MetHb}$$

The formation of methemoglobin from oxyhemoglobin by substances such as aminophenols, phenylhydroxylamine, phenylhydrazine and hydrazobenzene may be formulated in a similar manner.

A third important mechanism in the formation of methemoglobin from hemoglobin is autoxidation. Neill (184–187) found that various autoxidizable substances present in turpentine, linseed oil, the alcohol-soluble substance of potato juice, and sterile pneumococcus and other bacterial filtrates were capable of accelerating the formation of methemoglobin. For example, at an oxygen tension of 25 mm. of Hg in the presence of linseed oil, a 4.4 mM solution of hemoglobin yielded 1.1 mM of methemoglobin in two hours. This type of methemoglobin formation is greatly dependent upon the oxygen tension, is negligible at zero oxygen tension and maximal at relatively low tensions, about 25 mm. of Hg, where about half of the hemoglobin is in the form of oxyhemoglobin. At higher oxygen tensions, where a greater proportion of the hemoglobin is in the form of oxyhemoglobin, the rate of formation of methemoglobin is again decreased. It may be noted that spontaneous conversion of hemoglobin to methemoglobin takes place to an appreciable extent in drawn blood or in laked cells. Presumably, this is due to the presence of autoxidizable substances present in blood, for it does not occur when purified crystallized hemoglobin is exposed to air or oxygen.

The dependence of autoxidation of hemoglobin upon the oxygen tension has been formulated more precisely by Brooks (29–31) as follows:

$$\frac{dx}{dt} = k\,[\alpha(a - x)]\,\frac{\mathrm{bp}_{\mathrm{O}_2}}{1 + \mathrm{bp}_{\mathrm{O}}}$$

where x is the concentration of methemoglobin, a is the initial concentration of hemoglobin, α is the ratio of hemoglobin to total hemoglobin, and k and b are constants. Legge (153) has interpreted this equation to indicate the spontaneous decomposition of an intermediate, $Hb_4(O_2)_2$, to form methemoglobin.

C. *Physical properties of methemoglobin*

1. *Spectrophotometric absorption.* One of the most important properties of methemoglobin and one which is utilized in its quantitative estimation is its spectral absorption. The reader will recall that the capacity of a substance to transmit light of various wave lengths may be expressed by the extinction coefficient, $E^{1\%}_{1\,\mathrm{cm.}}$, which represents the ratio of the logarithm of the incidental light, I, to the transmitted light, T, of a 1 per cent solution through a 1 cm. thickness.[1] The ratio can be very easily estimated by determining the transmissions of an appropriate blank solution and a solution of the substance under test. The dilution of the latter must be chosen so that it can be read at a convenient range on the photometer; appropriate calculations to obtain the transmission of a 1 per cent solution through a 1 cm. thickness can then be made. It is also possible to express the extinction as the molar extinction coefficient, E_{mol}, where the concentration of the substance is 1 mole per liter or as the millimolar extinction coefficient, E_{mM}, where the concentration is 1 millimol per liter.

The absorption spectrum of methemoglobin has been the concern of many investigators since the identity of this compound was first established. More recent investigations are those of Hari (93), of Austin and Drabkin (8, 9), of Urbain and Greenwood (230), and of Holden (124). It has been repeatedly noted that the absorption spectrum depends upon the pH. The nature of these changes can perhaps best be illustrated by the work of Urbain and Greenwood (230) in the visible range of 500 to 660 mμ. For example, the values of E_{mM} at 500 mμ decrease from 8.0 at pH 1.0 to 6.9 at pH 2.5, rise to 9.6 at pH 6.0 and thereafter begin to decrease again. At a wave length of 630 mμ, the value of E_{mM} is 2.4 at pH 1.0, decreases to 2.0 at pH 2.5, then rises to 3.9 at a pH range of 6.0 to 7.0. However, in general, the variation of the value of E_{mM} with pH was different at different wave lengths.

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[1] This and subsequent units of extinction may also be designated by the Greek letter, ϵ.

The change in the absorption spectrum has received various explanations. Austin and Drabkin (9) postulated the existence of two forms of methemoglobin, acid and alkaline, which had different absorption characteristics and which were related by the following equation:

$$\mathrm{pH} = 8.12 + \log \frac{[\mathrm{MHb\ alkaline}]}{[\mathrm{MHb\ acid}]}$$

According to this equation, a 50-50 per cent mixture would be present at pH 8.12, and at pH 9.1 about 91 per cent of methemoglobin would exist in the alkaline form. Holden (124) postulates the existence of alkaline methemoglobin down to pH 6.3, with denaturation of methemoglobin setting in at pH 3.9. Urbain and Greenwood (230) have assumed the existence of several components in addition to alkaline and acid methemoglobins. As the acidity is increased the changes which occur may be conceived as follows: from pH 9.0 to 6.0, a shift from alkaline methemoglobin to acid methemoglobin; from pH 6.0 to 4.5, a shift from acid methemoglobin to some third compound; from pH 4.5 to 2.75, a shift to a compound such as acid hematin; below 2.75, another undefined compound or colloidal substance is formed. Lemberg and Legge (153) have submitted summaries of the values in the literature, on the assumption that there are only two forms of methemoglobin, acid and alkaline:

Acid methemoglobin		
Maxima, mμ	630	500
E_{mM}	3.7–3.8	9.5
Alkaline methemoglobin		
Maxima, mμ	577	540
E_{mM}	8.5	9.7

The absorption of methemoglobin has also been studied in the infra-red and ultraviolet regions. Holden (119, 126) obtained for acid methemoglobin at pH 4.68 a band at 407 mμ with an E_{mM} of 154. For alkaline methemoglobin at pH 10.8, the absorption band occurred at 411 mμ with an E_{mM} value of 90. Horecker (127) did not observe any distinctive absorption bands for methemoglobin in the infra-red region of 700 to 1000 mμ. At pH about 8.9, the value of E_{mM} rose from about 0.30 at 685 mμ to 0.53 at 818 mμ, then decreased to 0.26 at 940 mμ. At pH about 6.3 to 6.7, the value of E_{mM} rose from 0.15 at 700 mμ to about 0.79 at 980 mμ.

2. *Magnetochemical properties.* The magnetic properties of methemoglobin as

well as of related blood pigments have received considerable attention (54, 55, 57, 65, 227). When a substance is placed in a magnetic field, the electrons are so accelerated that a polarization results. The polarization is, of course, opposite to that of the applied field; the substance as a whole tends to be repelled by a magnet and hence the substance is said to be diamagnetic. In contrast, there are other substances which, although rendered diamagnetic when placed in a magnetic field, are also inherently paramagnetic, that is, they are attracted by a magnet. The paramagnetic characteristic of a substance is associated with the presence in these substances of atoms, ions or molecules which contain unpaired electrons. Indeed, the paramagnetic polarization is due to the spin magnetic moments of these electrons and to the moments of their orbital motion.

The principle underlying the measurement of paramagnetic susceptibility is that of "magnetic pull balanced by gravitational pull." Essentially a vertical glass cylinder containing the substance under test is suspended from one arm of a balance and the weight is measured before and after the application of a strong magnetic field to one end of the cylinder. The difference in weight is due to the magnetic field acting on the cylinder. In practice, a cylindrical tube is divided into an upper and lower chamber by a glass partition. The upper chamber is filled with the substance under test, and the lower chamber with pure solvent or with a diamagnetic substance. In the case of hemoglobin derivatives, the establishment of the diamagnetism of carboxyhemoglobin and the determination of the absolute paramagnetic susceptibility of ferrohemoglobin ($12{,}430 \times 10^{-6}$ c.g.s.u.) permitted the use of the following equation:

$$\chi_m = \frac{\Delta w - \Delta w_{\mathrm{COHb}}}{\Delta w_{\mathrm{Hb}} - \Delta w_{\mathrm{COHb}}} \times 12{,}430 \times 10^{-6} \text{ c.g.s.u.}$$

where Δw, Δw_{Hb} and Δw_{COHb} are the forces measured, in milligrams, for the solution under investigation, for ferrohemoglobin and for carboxyhemoglobin; χ_m is the molal magnetic susceptibility.

The value of the molal magnetic susceptibility of methemoglobin has been investigated in great detail by Coryell, Stitt and Pauling (57) at varying pH levels and ionic strengths. In general, the values for χ decreased from values of about $14{,}000$ to $15{,}000 \times 10^{-6}$ c.g.s.u. units in a pH range of about 5 to 7, to a value of about $8{,}000 \times 10^{-6}$ c.g.s.u. units at a pH of about 11 to 12. Substitution of the molal magnetic susceptibility χ_m in the equation $u = 2.84 \sqrt{\chi_m T}$ where T is the absolute temperature yields a value for u of 5.80 Bohr magnetons for methemoglobin and one of 4.47 Bohr magnetons for methemoglobin hydroxide. This indicates that there are five unpaired electrons per heme (ionic bands) in methemoglobin, and three in methemoglobin hydroxide.

3. *Other physical properties.* The crystallization of methemoglobin has, of course, permitted more precise studies of the chemical and physical properties of this substance. Methods for obtaining such crystalline preparations have been submitted by Levy (159) and by Dénes (63). These methods involve oxidation of reduced crystalline hemoglobin by an aqueous solution of potassium ferri-

cyanide in slight excess, or of crystalline oxyhemoglobin by an alcoholic or alkaline solution of the ferricyanide. The solubility characteristics of crystalline methemoglobin have been studied by Levy and the X-ray diffraction pattern has been investigated by Boyes-Watson and his associates (27) and by Perutz (195).

D. Chemical interactions and properties

Methemoglobin can combine with a great variety of substances. Most of these interactions consist of a combination of the ferric ion with other ions such as fluoride or cyanide, but changes in the globin part of the molecule are also possible.

1. *Methemoglobin hydroxide.* We have already discussed to some extent the interactions of methemoglobin with hydroxyl ion; at pH levels greater than 7, the hydroxyl ion becomes attached to the sixth bond position of the iron atom, and the spectrum becomes altered, with new maxima appearing at 577 and 540 mμ (153). The addition of sodium hydroxide to methemoglobin may also result in the denaturation of the globin moiety. As we have already noted (100), spectrophotometry yields a pK value of 8.1 for the interaction of methemoglobin with hydroxyl ion: MetHb + $H_2O \rightleftarrows$ MetHbOH + H^+. A similar value has been obtained by magnetochemical titration (57).

2. *Methemoglobin Fluoride.* Methemoglobin reacts with fluoride ion to form methemoglobin fluoride, also known as fluoro-methemoglobin (97, 100). Solutions of this compound are red, with absorption bands at 610 mμ and in the green part of the spectrum. Haurowitz (100) found a ratio of one atom of fluorine to one atom of iron in purified preparations of methemoglobin fluoride. Employing spectrophotometric methods, Lipmann (162) determined the value for the dissociation constant at neutral pH, $K = \frac{[MetHb]\ [F^-]}{[MetHbF]}$, to be about 0.015 M. However, the degree of dissociation varies with the pH and decreases in more acid regions. For example, Lipmann (162) observed that at 0.001 M concentration of sodium fluoride, the methemoglobin fluoride was in an undissociated form to the extent of 57 per cent at pH 3.8, of 27 per cent at pH 4.7 and of 6 per cent at pH 6.9.

The values for the dissociation constant of methemoglobin fluroide obtained by spectroscopic methods (105, 162) agree well with those obtained by Coryell and his associates (57), by means of magnetochemical methods. The latter derived the expression

$$\log K_{\text{MetHbF}} = -2.23 + 0.59\sqrt{\mu}$$

where u is the ionic strength. At a value of u equal to 0.5, log K equals $-2.23 +$ 0.4 or -1.8. K is therefore about 0.015 and in good agreement with the value Lipmann obtained on a solution of swine methemoglobin of unstated ionic strength (162).

3. *Cyanmethemoglobin.* The interaction of methemoglobin with cyanide is of considerable interest since it is this compound which has been considered to underlie the therapeutic effectiveness of methemoglobin in counteracting the

toxic or even lethal action of cyanide (35, 131, 181, 194). As we shall presently see, this interaction is also an important stage in the quantitative determination of methemoglobin. The product has been variously designated as cyanmethemoglobin, ferrihemoglobin cyanide, or hemiglobin cyanide (153). It has also been erroneously referred to as cyanhemoglobin, a term which should more properly be reserved for the possible existence of a compound of hemoglobin with cyanide (68). Cyanmethemoglobin was discovered by Kobert in 1891 (151). Zeynek (246) crystallized this compound and found that it contained one atom of cyanide per atom of iron.

The study of the interaction of methemoglobin with cyanide has been somewhat confused by the fact that potassium or sodium cyanide has usually been employed as the source of cyanide ion. Since the pK value of hydrocyanic acid is 9.1, these salts give an extremely high concentration of hydroxyl ion and hence provide the conditions for the formation of methemoglobin hydroxide as well as of cyanmethemoglobin. It is, therefore, necessary to consider the role of pH in the interaction of methemoglobin with cyanide ion. The various aspects of this interaction have been formulated as follows by Lemberg and Legge (153):

$$MetHb + HCN \rightleftarrows MetHbCN + H^+ \cdots a$$
$$MetHbOH + HCN \rightleftarrows MetHbCN + H_2O \cdots b$$
$$MetHbOH + CN^- \rightleftarrows MetHbCN + OH^- \cdots c$$

Reactions *a* and *c* are sensitive to pH while reaction *b* is not.

The absorption spectrum of cyanmethemoglobin has been studied by a number of investigators (8, 73, 99). The outstanding feature of the spectrum of cyanmethemoglobin is a marked absorption band at 540 mμ. This characteristic is revealed most obviously and simply when a solution of carefully neutralized cyanide is added to methemoglobin; the color changes abruptly from brown to a bright red.

The formation of cyanmethemoglobin is the basis of the therapeutic and prophylactic actions of certain compounds in cyanide poisoning (35, 43, 131, 181, 194). The mechanism of this antidotal action may be formulated in terms of the following competing equilibria:

(1) Ferricytochrome oxidase + cyanide ion
⇄ ferricytochrome oxidase cyanide

(2) Methemoglobin + cyanide ion ⇄ cyanmethemoglobin

In accordance with these equilibria, the presence of methemoglobin in the blood of an animal, *before* cyanide is introduced, should decrease the toxicity of this agent. On the other hand, the production of methemoglobinemia *after* the administration of a lethal dose of cyanide may result in the liberation of free ferricytochrome oxidase from its combination with cyanide, and in the restoration of the normal sequence of oxidation-reduction reactions in the tissues.

Chen and his coworkers (43) developed a treatment for cyanide poisoning which consisted of the intravenous injection of sodium nitrite and sodium thiosulfate. The sodium nitrite led to the production of methemoglobin and the sodium thiosulfate helped in the elimination of cyanide through the production of thiocyanate. Jandorf and Bodansky (133) showed that when methemoglobinemia was induced in dogs by means of p-aminopropiophenone, the degree of protection against subsequent inhalation of hydrocyanic acid was proportional to the degree of methemoglobinemia. Albaum, Tepperman and Bodansky (3) studied the *in-vitro* competition between methemoglobin and cytochrome oxidase in brain homogenates for cyanide ion. It was shown that methemoglobin can reverse the cyanide inhibition of cytochrome oxidase activity.

4. *Hydrogen peroxide methemoglobin.* Im 1900 Kobert (152) showed that when hydrogen peroxide was added to a solution of acid methemoglobin, the color turned from brown to red and the absorption spectrum of methemoglobin was replaced by three bands at 500–513 mμ, 545–558 mμ and 584–600 mμ. Kobert reported that when the solution was allowed to stand, the brown color of methemoglobin reappeared. Haurowitz (99) found that hydrogen peroxide reacted more rapidly with acid than with alkaline methemoglobin and that hydroxyl and cyanide ion could displace the hydrogen peroxide from its combination with methemoglobin. Keilin and Hartree (145) observed that the addition of hydrogen peroxide to a solution of pure methemoglobin at a pH of 5.8 to 6.5 caused the appearance of two new absorption bands, namely, at about 589 mμ and 545 mμ. The band at about 500 mμ noted by previous investigators is due to unchanged methemoglobin. This interaction requires one molecule of H_2O_2 per atom of methemoglobin iron. Ethyl hydrogen peroxide forms a similar compound with methemoglobin with absorption bands at the same place in the spectrum (145).

Hydrogen peroxide methemoglobin is not a stable compound. On standing in solution, it rapidly reverts to methemoglobin (98, 145); for example, about 70 per cent free methemoglobin is found after thirty minutes of standing and about 87 per cent after sixty to seventy minutes. Para-phenylenediamine, benzidine, traces of sodium hyposulfite, but not catalase, greatly accelerate the reaction; neither oxygen nor hydrogen peroxide is evolved in the course of this breakdown. This appears to be due to a peroxidase-like activity of methemoglobin; the hydrogen peroxide is used in the oxidation of substances such as p-phenylenediamine or even methemoglobin itself. In this connection, it may be noted that when catalase is absent from a solution of oxyhemoglobin or when catalase activity is suppressed by the addition of small amounts of azide or cyanide, then hydrogen peroxide causes the formation of methemoglobin. It may be appreciated that the addition of hydrogen peroxide to oxyhemoglobin may lead to a succession of reactions in equilibrium, namely, the formation of methemoglobin, the combination of methemoglobin with hydrogen peroxide, the reversion to methemoglobin itself as well as the partial oxidation of methemoglobin, through a peroxidase reaction, to other compounds.

The nature of the oxidation products of methemoglobin caused by hydrogen peroxide has been of considerable interest in connection with a study of the nature of bile pigments. Lemberg and his associates (155) have shown that the green pigment, choleglobin, can be formed by the action of hydrogen peroxide directly on methemoglobin or even indirectly from hemoglobin. Holden has similarly obtained a green basic compound of the bile-pigment type, a haematin and a porphyrin (123, 125).

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5. *Methemoglobin azide.* In the course of an extensive study of the physiological actions of azide, Smith and Wolff (219) in 1904 observed that this ion formed a definite compound with methemoglobin, similar to that which cyanide ion formed. Keilin (143) found that the addition of sodium azide to methemoglobin at a pH of 5.6–6.4 caused a change to a red-colored solution with the appearance of two absorption bands at 575.0 mμ and 542.5 mμ. The dissociation constant of methemoglobin azide is about 1.2×10^{-4} and the compound contains 1 molecule of azide per atom of methemoglobin iron. The mean value of the magnetic susceptibility is consistent with the possibility that this compound has one unpaired electron per iron atom, with covalent bonds (56).

6. *Methemoglobin hydrosulfide.* Hydrogen sulfide reacts with methemoglobin at an acid pH, 5.6–5.8, to form methemoglobin hydrosulfide, a red-colored compound which has absorption bands at 545 mμ and 578 mμ. Keilin (144) found that the dissociation constant of this compound was about 1.3×10^{-6}. Coryell and his associates (57) observed that methemoglobin hydrosulfide underwent rapid auto-reduction to ferrohemoglobin, a phenomenon not mentioned by Keilin. In spite of the rapid breakdown of the hydrosulfide, Coryell (57) obtained values for the magnetic susceptibility which indicated the existence of one unpaired electron per iron atom, with covalent bonds.

7. *Nitric oxide methemoglobin.* There has been considerable controversy concerning the combination of nitric oxide with methemoglobin. Haurowitz (100) reported that nitric oxide combined only with reduced hemoglobin in the complete absence of oxygen, and that methemoglobin was reduced by nitric oxide to hemoglobin. In contrast, Anson and Mirsky (6) considered that nitric oxide oxidized hemoglobin to methemoglobin, and that the latter compound then reacted with additional nitric oxide. Keilin and Hartree (146) reconciled these two opposing viewpoints by showing that reduced hemoglobin when mixed with nitric oxide formed a red-colored solution with absorption bands at 536 mμ and 574.5 mμ. Methemoglobin, upon reaction with nitric oxide, yielded a solution with absorption bands at 568 mμ and 531 mμ. The nitric oxide-methemoglobin compound is unstable and reverts rapidly to methemoglobin.

8. *Other compounds of methemoglobin.* Methemoglobin has been shown to combine with a number of other substances, namely, fulminate (15), thiocyanate (140), cyanate (109, 214), ethanol (56), ammonia (56), imidazole (204), and oxidation products of aniline (135). The interaction of acid methemoglobin with thiocyanate is not sensitive to pH; the pK value of this reaction is 3.14. The interaction of alkaline methemoglobin with thiocyanate is pH dependent, and the pK value is 9.8. The magnetic susceptibilities of the compounds formed with ethanol and with ammonia have been determined by Coryell and Stitt (56). Imidazole methemoglobin has one unpaired electron and hence covalent bonds. This compound is not a very firm one; the pK value is 2.7 at pH 7 (204).

E. Oxidation-reduction equilibrium between hemoglobin and methemoglobin

In addition to the reactions of methemoglobin which we have described above, it is necessary to consider the oxidation-reduction equilibrium between hemoglobin and methemoglobin. As we shall show subsequently, methemoglobin is

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E. *Oxidation-reduction equilibrium between hemoglobin and methemoglobin*

In addition to the reactions of methemoglobin which we have described above, it is necessary to consider the oxidation-reduction equilibrium between hemoglobin and methemoglobin. As we shall show subsequently, methemoglobin is reduced *in vivo* to hemoglobin by a variety of substances. Reduction *in vitro* can also be accomplished by a number of compounds such as ascorbic acid (236), reduced diphosphopyridine nucleotide (89), and the leuko forms of dyes (177).

The oxidation of hemoglobin to methemoglobin and the reduction of methemoglobin to hemoglobin raise the question whether this is a reversible system and, if so, what its oxidation-reduction potential is. This aspect has been investigated in some detail by Conant and his associates (51–53). After some attempts which yielded approximate values, Conant was able to make fairly precise measurements of the potentials on crystallized horse methemoglobin against an inert platinum electrode, obtaining a value of 152 ± 5 millivolts for E_0 at pH 7.0, in the electrochemical equation

$$E = E_0 + \frac{RT}{n} \ln \frac{[\mathrm{MHb}]}{[\mathrm{Hb}]}$$

where E_0 represents the potential of an equimolar mixture of hemoglobin and methemoglobin. The ionic strength, u, in these experiments was 0.3. Further determinations at a higher ionic strength, $u = 1.6$, yielded a somewhat lower value, about 29 millivolts for E_0 at pH 7.0. Employing a spectrophotometric method, Conant and Scott (53) obtained a value of 150 millivolts at pH 6.9, essentially in agreement with the value obtained by the electrochemical method.

The oxidation-reduction potential of this system has also been investigated by a number of other workers (14, 104, 106, 108, 209–211), particularly with respect to the effect of pH. Barnard (14) also obtained a value of 153 millivolts for E_0 at pH 7.07 at a total concentration of dog methemoglobin of 3.52 mM per liter, and an increase of about 75 millivolts with each increase of one pH unit. Havemann and Wolff (108) studied the effect of pH upon the oxidation-reduction potential, found a value of about 95 millivolts at a pH of 7.0 and a change of about 70 millivolts per pH unit.

The oxidation-reduction potential of the methemoglobin-hemoglobin system has also been investigated by means of dye indicators. In this connection, it may be recognized that a dye with a relatively high oxidation-reduction potential reduces little methemoglobin, whereas dyes with low potentials convert methemoglobin to hemoglobin very effectively. For example, a 90 per cent reduced solution of indigosulfonate ($E_0 = -125$ millivolts) reduced methemoglobin completely, whereas under similar conditions a 90 per cent reduced dichlorphenolindophenol ($E_0 = +217$ millivolts) reduced only 70 per cent of the methemoglobin (209, 210). Essentially this method consists in determining the proportion of methemoglobin which is reduced in the absence of oxygen by mixtures of the reduced and oxidized forms of various dyes. The potentials of these dye mixtures can be calculated from the following equation:

$$E = E_0 + 0.03 \log \frac{\text{oxidized form of dye}}{\text{reduced form of dye}}$$

where E_0 is the potential of a 50–50 per cent mixture of these forms at pH 7.0.

The E_0 value of the methemoglobin-hemoglobin equilibrium may then be calculated as follows:

$$E_{O,\,(\mathrm{MetHb})/(\mathrm{Hb})} = E_{\mathrm{dye}} - 0.06 \log \frac{\mathrm{MetHb}}{\mathrm{Hb}}$$

Employing this equation, Schmidt (210) obtained a value for E_0 of 202 to 214 millivolts, which was higher than that recorded by others. Havemann and Heubner (106) contended that this value was erroneouly high. Schmidt had estimated the amount of reduced hemoglobin formed through the action of the dye by determining the amount of carbon monoxide that could be combined chemically with the hemoglobin-methemoglobin mixture. According to Havemann and Heubner (106) such a technique causes a displacement of the equilibrium, MetHb ⇄ Hb, to the right, and consequently the values for the amount of hemoglobin and the value of E_0 for the methemoglobin-hemoglobin system are too high.

It was appreciated by Conant and his associates (51–53) that the methemoglobin-hemoglobin system was to be classed among those sluggish oxidation-reduction systems in which stable potentials were not quickly established and periods ranging up to several hours were necessary for the electrode to come into equilibrium with the system. For example, Conant and Pappenheimer (52) found that, in their most satisfactory determinations, about half an hour was necessary for establishment of a stable equilibrium; on the other hand, there were many runs in which it was not possible to attain equilibrium, and the results had to be discarded.

The sluggishness and uncertainty which characterize the establishment of equilibrium values in the methemoglobin-hemoglobin system prompted Taylor and Hastings (228) to employ electromotively active mediators. For example, they observed that in the presence of 3.5×10^{-5} M m-toluylenediamine indophenol, a stable potential was established within fifteen minutes and remained constant for an hour; in contrast, under the same conditions but in the absence of this mediator, the potential was still decreasing after two hours. Employing this method, Taylor and Hastings obtained a value of $+139$ millivolts for E_0 at pH 7 and at a temperature of 30°. This method has been utilized by Havemann (104) in an extensive study of the influence of pH and temperature on the oxidation-reduction potential. At 30° and pH 7, Havemann obtained a value of $+150$ millivolts for E_0.

III. BIOCHEMISTRY OF METHEMOGLOBIN

This section will be concerned with the *in-vivo* equilibrium between hemoglobin and methemoglobin, the effect of methemoglobin on the oxygen-carrying properties of the blood, the methods of determining the concentration of methemoglobin in the blood, and, finally, the enzymic mechanisms involved in the reduction of methemoglobin.

A. Methods for quantitative determination of methemoglobin in blood

It may be of value to describe briefly some of the principles and chief features of the quantitative estimation of methemoglobin in the blood. Most of the methods which have been described may be classified into three main groups: (a) gasometric methods, (b) those based on the spectrophotometry before and after treatment of blood with cyanide which converts the methemoglobin into cyanmethemoglobin, and (c) direct spectrophotometric methods.

1. *Gasometric methods.* It has been known for a number of years that normal human blood contains a hemoglobin moiety which contains trivalent iron and which lacks the power to combine reversibly with oxygen or carbon monoxide until the ferric ion is reduced to ferrous ion (5, 188, 197, 233, 234). This moiety has been termed "inactive hemoglobin." Nicloux and Fontes (188) introduced a method for determining this portion of the blood pigment by reducing it with $Na_2S_2O_4$ and noting the increase in the carbon monoxide capacity. The procedure has been refined by Van Slyke and his associates (233, 234). Ramsay (197) used essentially the same principle but reduced the inactive hemoglobin with titanous ion (Ti^{+++}) and utilized the ensuing increase in oxygen-combining power as a measure of the "inactive pigment" originally present.

2. *Spectrophotometric methods.* These methods depend upon the decrease of transmission of light of wave length of 635 mμ or 800 mμ, usually the former, when cyanide is added to a solution of methemoglobin. As pointed out earlier in this paper, the cyanide changes the brownish methemoglobin to the red cyanmethemoglobin but does not affect hemoglobin. Procedures based on this principle have been described by Evelyn and Malloy (73), Havemann, Jung and von Issekutz (107), Michel and Harris (178), and Horecker and Brackett (128). That of Evelyn and Malloy (73) may be described in order to illustrate more specifically the principle which is involved. One volume of whole blood is delivered into one hundred volumes of M/60 phosphate buffer of pH 6.6 in a colorimeter tube. The solution is allowed to stand for five minutes and the optical density is read at 635 mμ in an Evelyn photoelectric colorimeter or other suitable spectrophotometer. One drop of a neutralized sodium cyanide solution is then added to convert any methemoglobin to cyanmethemoglobin, and another reading is made at 635 mμ after several minutes. The difference between the two readings is proportional to the concentration of methemoglobin; if the Evelyn photoelectric colorimeter is used the concentration may be expressed directly in Gm. per 100 cc. as follows:

$$M = \frac{100(L_1 - L_2)}{2.77}$$

Other photoelectric colorimeters or spectrophotometers require, of course, different factors in the denominator.

If the concentration of methemoglobin is desired as a percentage of the total blood pigment, it is not necessary to note precisely the amount of blood that is added to the phosphate buffer. The solution of blood is divided into two portions.

In one portion, the difference between the readings at 635 mμ is noted before and after the addition of neutralized cyanide. The second portion is treated with ferricyanide to convert all the blood pigment into methemoglobin, and readings are taken at 635 mμ before and after the addition of neutralized cyanide. The difference in the readings of the first portion divided by the difference in the readings of the ferricyanide-treated sample yields the value for the percentage of methemoglobin.

The amount of methemoglobin in methemoglobin-hemoglobin mixtures has also been measured by determining the ratio of the absorption at different wave lengths as, for example, that at 575 or 540 mμ to that at 560 mμ (67, 198). However, such methods have now been largely abandoned in favor of those which depend upon the conversion of methemoglobin to cyanmethemoglobin.

TABLE I

Concentration of inactive hemoglobin in normal human blood as determined by the gasometric method

INVESTIGATOR	NO. OF SUBJECTS	NO. OF BLOOD SPECIMENS	PER CENT OF TOTAL Hb		
			Minimum	Maximum	Mean
Ammundsen (4)	53	63	0	12.0	3.0
Kallner (141)	20	20	−1.2	1.2	−0.2
Roughton, Darling and Root (203)	4	26	0.7	5.0	2.8
Kallner (142)	4	4	−1.5	0.5	−0.5
Ramsay (197)	38	38	−0.5	7.0	1.9
Van Slyke and associates (234)	19	19	0.6	1.8	1.28

The question concerning the extent to which values obtained by the gasometric method agree with those obtained by the cyanide-spectrophotometric method is discussed in the following section.

B. Concentration of methemoglobin present in normal blood

Table I shows the values recorded by various investigators for the concentration of "inactive hemoglobin" present in normal human blood. It may be noted that values as high as 12 per cent have been reported, but that the average values are usually about 1 to 2 per cent of the total blood pigment. In Table II are shown the concentrations of methemoglobin normally present in human blood, as determined by the cyanide-spectrophotometric method. With the exception of a high value of 8 per cent recorded by Havemann, most values range between 0.0 and 2.4 per cent of the total blood pigment.

A comparison of the values listed in Table I and II shows that the concentration of "inactive hemoglobin" appears greater than that of methemoglobin as determined by the cyanide-spectrophotometric method. This difference was made particularly clear by the study of Van Slyke and his associates (234). It would, therefore, seem that the "inactive hemoglobin" contains some other component besides methemoglobin. Indeed, this component appears to be converted into active hemoglobin when blood is allowed to stand for several hours; in contrast, the concentration of methemoglobin does not decrease. Thus, in Van Slyke's work (234) the concentration of "inactive hemoglobin" as per cent of the total blood pigment was: 1.29 ± 0.35 per cent three minutes after being drawn from the vein; 0.90 ± 0.32 per cent, 0.5 to 1.5 hours later; 0.56 ± 0.23 per cent, two to four hours after venipuncture. In contrast, at these times the concentrations of methemoglobin, as determined by the cyanide-spectrophotometric method, were 0.41 ± 0.44 per cent, 0.44 ± 0.59 per cent and 0.33 ± 0.44 per cent, respectively.

It is of interest that methemoglobinemia is also normally present in animals. Indeed, in some species the extent of this normal methemoglobinemia appears to be greater than in normal man. Thus, Ramsay (197) found the "inactive hemoglobin" to range from 0 to 25.5 per cent and to average 7.0 of the total blood pigment in a series of seventeen horses. Issekutz (132), employing the more specific cyanide-spectrophotometric method, found that in cats the concentration of methemoglobin ranged from 4 to 17 and averaged 8.6 per cent of the total blood pigment. Heubner and his associates, however, have failed to note such large differences between the methemoglobin content of the blood of man and various animal species. Employing spectrophotometric methods based on carboxyhemoglobin as well as cyanmethemoglobin formation, Heubner (114) obtained the average values for five to seven individuals in each species, as shown in Table III.

TABLE II

Concentration of methemoglobin in normal human blood as determined by the cyanide-spectrophotometric method

INVESTIGATOR	NO. OF SUBJECTS	NO. OF BLOOD SPECIMENS	PER CENT OF TOTAL Hb		
			Minimum	Maximum	Mean
Havemann and associates (107)				8	
Schmidt-Burgh (208)	20	20	1.1	2.4	1.7
Paul and Kemp (191)	20	20	0.1	0.8	0.4
Van Slyke and associates (234)	19	19	0.0	1.1	0.4

C. The hemoglobin-methemoglobin equilibrium

The presence of methemoglobin in normal blood as well as other phenomena which we shall presently note raises the definite possibility that there normally exists in blood the equilibrium, methemoglobin ⇄ hemoglobin, which is regulated by various oxidizing and reducing substances, but which under ordinary conditions is shifted far to the right. Such a concept helps to explain the differing extents of formation of methemoglobin by the same drug in different species, the differing rates of reduction of methemoglobin in various species and the occurrence of idiopathic methemoglobinemia.

1. *Rate of disappearance of methemoglobin.* An overall view of the rate with which methemoglobin is reduced to hemoglobin *in vivo* may be determined experimentally in either of two ways: (a) by following the rate of reduction of methemoglobin of intravenously injected methemoglobinemic erythrocytes; (b) by following the rate of disappearance of methemoglobin after the injection of a methemoglobin-forming substance.

The former method has been employed by Heubner and Moebus (117), Jung (139) and Gelinsky (80). Jung removed 30 to 40 cc. of blood from cats, converted the hemoglobin to methemoglobin by means of an isotonic sodium chloride-sodium nitrite mixture, washed the resulting methemoglobin-containing erythrocytes several times with saline, made them up to the original volume with saline and reinfused them into the cat. The rate of conversion varied with the initial concentration of methemoglobin. For example, when sufficient methemoglobin-containing blood was infused so as to yield an initial concentration of about 3.8 grams per 100 cc., the concentrations at the end of 1, 2, 4 and 6 hours were, respectively, about 2.4, 2.1, 1.4 and 1.0 gram per 100 cc. When the initial concentration was about 1.3 grams per 100 cc., the corresponding concentrations at these intervals were about 0.9, 0.7, 0.5 and 0.2 gram per 100 cc. About eight hours were required to reduce an initial concentration of 5 grams of methemoglobin per 100 cc. of blood to negligible levels.

TABLE III

Concentration of methemoglobin in normal animals

SPECIES	AVERAGE TOTAL HEMOGLOBIN CONC.	AVERAGE METHEMOGLOBIN CONC.	METHEMOGLOBIN AS FRACTION OF TOTAL BLOOD PIGMENT
	Gms. per 100 cc.	*Gms. per 100 cc.*	*per cent*
Man	14.3	0.10	0.7
Dog	14.9	0.14	0.9
Cat	12.2	0.13	1.1
Rat	12.3	0.10	0.8
Rabbit	12.8	0.02	<0.2
Guinea pig	12.7	0.03	<0.2

The comparison of the rates of conversion of methemoglobin to hemoglobin in different species is of interest. In contrast to Jung's results in cats, Gelinsky (80) observed that a period of about four hours was required in rats and only about one hour in guinea pigs for the disappearance of an initial concentration of 5 grams of methemoglobin per 100 cc.

The second method of studying the rate of conversion rests on the assumption that, although both methemoglobin-forming and methemoglobin-reducing reactions take place concurrently and the concentration of methemoglobin at any moment is the resultant of these two groups of reactions, the methemoglobin-forming reactions become negligible after a stated period in the case of certain compounds and that the apparent rate of disappearance of methemoglobin is practically identical with its true rate of disappearance.

Cox and Wendel (58) tested twenty-five compounds for their methemoglobin-producing potency in dogs. After the attainment of the maximum concentration, the rate of disappearance was constant and amounted to about 11 per cent of the total blood pigment per hour. This value held whether the maximal concentration was 60 per cent of the total pigment, as with p-aminophenol and sodium nitrite, or about 25 per cent as was the case after injection of hydroxylamine. Such constancy of rate of conversion of methemoglobin had not been observed by Jung (139). In view of the paucity of data in this field, it is difficult to reconcile this conflict. It is possible that difference in technic or difference in species used may account for the discrepancy.

The comparative rates of reduction in infused and drug-induced methemoglobinemias have recently been considered in detail by Kiese (147). He injected intravenously 25 mg. of sodium nitrite per kg. into a dog and obtained a maximal methemoglobin concentration of about 5.2 grams per 100 cc., about 35 per cent of the total pigment. The rate of reduction was about 1.5 gram per 100 cc. per hour or about 10 per cent per hour, a value in essential agreement with that of Cox and Wendel (58). When blood was removed from the animal and an equal volume of methemoglobin-containing cells was then infused, the rate of reduction was 1.3 grams per 100 cc. or about 8.5 per cent per hour. However, since only about 40 per cent of the cells or about two million were methemoglobinemic, Kiese (147) assumed that the corrected rate of reduction should be $1.3 \times \frac{5}{2}$ or 3.2 grams of methemoglobin per hour.

It would thus appear that the rate of methemoglobin reduction is more rapid in methemoglobinemias induced by the infusion of methemoglobinic blood than in those that are drug induced. A possible explanation for this is that, even in a rapidly induced methemoglobinemia, the rate of reduction appears slower than is actually the case because of the continued presence of methemoglobin-forming material. This view is substantiated by the finding of Greenberg, Lester and Haggard (88) that in nitrite-induced methemoglobinemia in rats when the concentration of methemoglobin was maximal, nitrite was still present in appreciable amounts.

2. *Factors controlling rate of disappearance of methemoglobin.* The factors which may influence the rate of reconversion of methemoglobin to hemoglobin have received considerable attention. Brooks (32) reported that intravenous injection of glucose accelerated greatly the disappearance of nitrite-induced methemoglobinemia in rabbits; on the other hand, Cox and Wendel (58) found no such influence on nitrite-induced methemoglobinemias in the dog either when the blood glucose was elevated as the result of glucose infusion or decreased as the result of insulin injection. Cox and Wendel also observed that lowering the body temperature decreased the rate of methemoglobin disappearance. Of chief interest in this connection are those compounds which reduce the methemoglobin rapidly when they are injected or, in some instances, when they are fed. Among these are methylene blue and other oxidation-reduction dyes (49), ascorbic acid, 2-3 dimercaptopropanol, glutathione or cysteine (153). We shall discuss later the possible therapeutic implications of the substances which cause

globin-producing potency in dogs. After the attainment of the maximum concentration, the rate of disappearance was constant and amounted to about 11 per cent of the total blood pigment per hour. This value held whether the maximal concentration was 60 per cent of the total pigment, as with p-aminophenol and sodium nitrite, or about 25 per cent as was the case after injection of hydroxylamine. Such constancy of rate of conversion of methemoglobin had not been observed by Jung (139). In view of the paucity of data in this field, it is difficult to reconcile this conflict. It is possible that difference in technic or difference in species used may account for the discrepancy.

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the reversion of methemoglobin to hemoglobin. The elucidation of the mechanisms which are involved in the *in-vivo* reversion of methemoglobin to hemoglobin has gained much from *in-vitro* studies.

Warburg, Kubowitz and Christian (243) observed that, when methemoglobin-containing red cells were allowed to incubate in the presence of glucose, hexosemonophosphate, hexosediphosphate or lactic acid, methemoglobin was changed to hemoglobin and oxygen was absorbed. For example, the oxygen consumption of 2 cc. of normal rabbit erythrocytes in two hours in the presence of 0.1 cc. of 10 per cent glucose was 8 cmm. The oxygen consumption of 2 cc. of approximately 50 per cent methemoglobinized cells was 68 cmm. in the absence of glucose, and 238 cmm. in the presence of 0.1 cc. of 10 per cent glucose.

It may be stressed that no reduction of methemoglobin occurs when methemoglobinemic erythrocytes are washed free of glucose. Drabkin (66) showed that the reduction of methemoglobin in erythrocytes to hemoglobin was accompanied by a decrease in glucose. The reversion of methemoglobin to hemoglobin was inhibited by hemolysis, or by the addition of fluoride or iodoacetate. The inhibition by fluoride was overcome by means of the addition of pyruvate, but not of lactate.

These findings would indicate that one or more enzyme systems in the erythrocyte are involved in the reduction of methemoglobin to hemoglobin, that the existence of this enzyme system is dependent upon the integrity of the erythrocyte and is associated with the glycolytic process. Warburg and Christian (240) isolated a macromolecular "zwischenferment" and a coenzyme of low molecular weight which, together with hexosemonophosphate, was capable of reducing methemoglobin.

The nature of the enzyme systems which were capable of reducing methemoglobin prepared directly from crystallized horse hemoglobin has been studied by Kiese (148, 149). Employing the uptake of carbon monoxide as a measure of the formation of hemoglobin, he found that purified methemoglobin was reduced directly by glyceraldehyde, ascorbic acid or cysteine; the rate of reduction was dependent upon the concentrations of these substances and of methemoglobin and upon the pH. When the coenzyme, triphosphopyridine nucleotide (TPN), isolated by Warburg, Christian and Griese (241) from red blood cells, was incubated with methemoglobin and hexosemonophosphate, it was observed that the rate of reduction was much slower than in the intact erythrocyte. Kiese assumed that an additional enzyme factor in the erythrocyte was necessary for the reduction of methemoglobin and found that an extract of erythrocytes in sufficiently high concentration markedly accelerated the conversion to hemoglobin. The isolation of the enzyme factor was accomplished by treating horse erythrocytes with toluene and carbon monoxide. Considerable hemoglobin separated out at this step and again when the resulting solution was treated at 0° with potassium phosphate at a pH of 7.0. The precipitated hemoglobin was centrifuged and washed with M phosphate solution of pH 7.0. These washings and the supernatant solution were combined and precipitated fractionally with ammonium sulfate. The fraction, precipitated between 20 and 40 per cent am-the reversion of methemoglobin to hemoglobin. The elucidation of the mechanisms which are involved in the *in-vivo* reversion of methemoglobin to hemoglobin has gained much from *in-vitro* studies.

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The role of the pyridine nucleotides in the reduction of methemoglobin was also stressed by Gutmann, Jandorf and Bodansky (89) who studied the well-known accelerative effect of methylene blue in the reduction of methemoglobin. The lack of reduction of methemoglobin in hemolyzed, methemoglobinemic blood raised the possibility that pyridine nucleotides were absent; there is evidence that pyridine nucleotides disappear rapidly upon the lysis of erythrocytes or the homogenization of animal tissues. Mann and Quastel (170) as well as Handler and Klein (91) had found that the addition of nicotinamide inhibits the disappearance of the pyridine nucleotides, presumably by inhibiting their enzymic hydrolysis. Applying this finding, Gutmann, Jandorf and Bodansky (89) observed that, whereas no reduction of methemoglobin occurred in hemolysates containing methylene blue and hexosediphosphate or lactate, appreciable reduction took place when nicotinamide was added. Much more direct proof of the role of the pyridine nucleotides was obtained when it was found that the reduced but not the oxidized form of diphosphopyridine nucleotide (DPN) reduced purified methemoglobin. This acceleration was increased greatly by the addition of methylene blue. It appeared from these experiments and the work of other investigators that the production of reduced pyridine nucleotides was an essential link in the reduction of methemoglobin. The removal of substrate or the inhibition of glycolysis by fluoride or iodoacetate interferes with the production of DPN-H_2 or TPN-H_2; hemolysis results in the destruction of these coenzymes in the red cells.

The study of the rare disease, familial idiopathic methemoglobinemia, has also contributed to our knowledge of the enzyme mechanisms involved in the conversion of methemoglobin to hemoglobin. In this disease, as the name indicates, methemoglobinemia is present in an individual from birth without apparent external cause. Lian and his coworkers (160) in 1939, as well as Barcroft and his associates (11) some years later, observed that in contrast to what occurs in the blood from normal individuals in whom methemoglobinemia has been induced the shed blood from persons with idiopathic methemoglobinemia shows little if any tendency for methemoglobin to disappear when the blood is allowed to stand. Sievers and Ryon (218) found that this stability of methemoglobin was not dependent in any way on the plasma, since plasma from a patient with the disease did not influence the rate of reduction of methemoglobin in normal human erythrocytes nor did normal plasma accelerate the reduction of methemoglobin in the erythrocytes of the patient.

As has already been noted, washed methemoglobinemic erythrocytes from normal persons show very little reversion of the methemoglobin to hemoglobin; such reversion takes place in the presence of added glucose or lactate (82, 84, 89). However, the presence of these substrates is not adequate for the reduction of methemoglobin in the erythrocyte from the individual with idiopathic methemoglobinemia (84).

According to Gibson (84), methemoglobin reduction in normal erythrocytes takes place through the oxidation of triosephosphate and lactate. This reaction requires, in addition to the production of these substrates from glucose, dehydrogenase, coenzyme I and coenzyme factor I or diaphorase. It is diaphorase which appears to be deficient in the erythrocytes of patients with familial idiopathic methemoglobinemia. Gibson found that the amount of this factor in the bloods of five patients was lower than the amounts in a series of bloods from twelve normal persons.

However, there are other aspects which still remain to be explored in order to furnish a complete picture of the enzymic mechanisms involved in the reduction of methemoglobin. For example, Spicer and his associates (221) have recently tested a variety of substrates for their ability to reduce methemoglobinemic erythrocytes of the dog at neutral pH. Only four hexoses, namely, D-fructose, D-glucose, D-mannose and D-galactose were found effective. In addition to the established effect of lactate, fumarate and L-malate were also found capable of reducing methemoglobin. The effects varied with the concentration of the substrates. Phosphate ion was necessary for a maximal response with glucose, mannose or fructose but not with galactose. Oxalate and citrate inhibited the reduction of methemoglobin by glucose, but citrate ion accelerated the reduction by lactate.

D. Effect of methemoglobinemia on the oxygen dissociation curve

The quantitative aspects of the combination between hemoglobin and oxygen have been well explored (1, 64, 120, 130, 192). It is appreciated that when the oxygen tension is plotted against the degree of oxygenation (or per cent of oxyhemoglobin formed) a so-called dissociation or sigmoid curve is obtained. Mathematical expressions to describe the shape of this curve and hence to formulate a general expression for the affinity between oxygen and hemoglobin have been submitted by a number of workers such as Hüfner (130), Hill (120), Adair (1), and Pauling (192). The affinity of oxygen for hemoglobin is influenced by temperature, pH, ionic strength, concentration of hemoglobin and, what is of particular relevance for the subject of this review, by the presence of other blood pigments such as carboxyhemoglobin and methemoglobin. We shall first briefly present the studies concerning the effect of carboxyhemoglobin, since it illustrates the effect of methemoglobin and more data concerning it are available.

In 1912, Douglas and his coworkers (64) showed that the presence of carboxyhemoglobin shifted the dissociation curve of the residual hemoglobin to the left. This observation was confirmed by subsequent workers and it was also demonstrated that the form of the dissociation curve became more hyperbolic in the presence of carboxyhemoglobin (202, 223). A quantitative idea of these effects may be obtained from the following tabulation of the oxygen dissociation of the *residual* oxyhemoglobin in the presence of various amounts of carboxyhemoglobin (Table IV). The values have been interpolated from the curves presented by Roughton and Darling (202).

It must be recognized, however, that the effects of poisoning by carbon monoxide are twofold. Not only is some of the hemoglobin combined with carbon monoxide and therefore becomes unavailable for transport of oxygen, but as pointed out above, the residual oxyhemoglobin becomes less capable of dissociation. This again may be demonstrated by the tabulation of the following data obtained from curves submitted by Roughton and Darling (Table V). That the presence of carboxyhemoglobin makes the dissociation curve of the residual oxyhemoglobin less sigmoid and more hyperbolic may be seen from a comparison of the last two columns. Thus a person with 40 per cent of his hemoglobin left uncombined with carbon monoxide shows about the same absolute amount of oxyhemoglobin as an anemic person with 40 per cent hemoglobin at 100 mm. Hg oxygen tension, but about 25 per cent more at 40 mm. Hg, 100 per cent more at 20 mm., and 280 per cent more at 10 mm. oxygen tension. Again, Table V shows that the individual with a 60 per cent carboxyhemoglobinemia has only 40 per cent as much oxyhemoglobin as the normal individual at an oxygen tension of 100 mm., but 160 per cent as much at a tension of 10 mm.

TABLE IV

Oxyhemoglobin dissociation in presence of carboxyhemoglobin

OXYGEN TENSION	PER CENT OF HEMOGLOBIN AS OXYHEMOGLOBIN IN PRESENCE OF		
	No COHb	20% COHb	60% COHb
mm. Hg			
10	13	21	45
20	36	49	73
30	58	70	87
40	74	82	95
60	90	93	

TABLE V

Effect of carboxyhemoglobin and anemia on the form of the dissociation curve of oxyhemoglobin

OXYGEN TENSION	OXYGEN BOUND TO HEMOGLOBIN (VOLUMES PER CENT) IN PRESENCE OF		
	No COHb	60% COHb	Anemia 40% of normal
mm. Hg			
10	2.4	3.8	1.0
20	7.0	5.9	2.8
30	11.7	7.0	4.8
40	15	7.6	6.0
60	18	7.9	7.2
100	19.3	8.0	7.8

That methemoglobin produces a similar effect on the dissociation of oxyhemoglobin was demonstrated by Darling and Roughton in 1942 (60). Quantitatively, boxyhemoglobin (Table IV). The values have been interpolated from the curves presented by Roughton and Darling (202).

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100	19.3	8.0	7.8

That methemoglobin produces a similar effect on the dissociation of oxyhemoglobin was demonstrated by Darling and Roughton in 1942 (60). Quantitatively, however, the effect of methemoglobin is less than that of carboxyhemoglobin. For example, a content of 23 per cent carboxyhemoglobin shifted the dissociation curve of the residual oxyhemoglobin in human blood about as far to the left as did a content of 43 per cent methemoglobin. The effect of methemoglobin on the oxygen dissociation curve is wholly reversible. Eder and his associates (69) have recently reported that the position and shape of the dissociation curve are not altered in familial idiopathic methemoglobinemia.

The alteration of the shape and position of the oxygen dissociation curve in carboxyhemoglobinemia or methemoglobinemia implies that the tissues are liable to anoxia, not only because of loss of the oxygen-carrying capacity of the blood, but also because the residual oxyhemoglobin is less capable of dissociating and therefore unloading oxygen in the tissues. We shall presently consider this as well as other physiological aspects of methemoglobinemia in greater detail.

The question naturally arises why carboxyhemoglobinemia or methemoglobinemia shifts the dissociation curve of oxyhemoglobin to the left and renders it less sigmoid and more hyperbolic. As we have already noted, a number of equations have been submitted for the dissociation of oxyhemoglobin itself. Although each of these does not offer a completely quantitiative explanation, the sigmoid shape of the curve is best understood by the intermediate compound hypothesis submitted by Adair (2) and later modified by Pauling and his associates (55, 192). According to this formulation, the blood hemoglobin molecule reacts with O_2 in four stages, one molecule combining stepwise with each of the four ferrous atoms, as follows:

$$Hb_4 + O_2 = Hb_4O_2\,;\ Hb_4O_2 + O_2 = Hb_4O_4\ ;$$
$$Hb_4O_4 + O_2 = Hb_4O_6\,;\ Hb_4O_6 + O_2 = Hb_4O_8$$

At very low O_2 pressures the principal compound formed would be Hb_4O_2, and the form of the dissociation curve in this region is a straight line. The greater ease of formation of the dioxy-compound, Hb_4O_4, is reflected in the upward turn of the curve, and the tapering off of the curve is due to the formation of the trioxy- and tetraoxy-compounds. Each of the four reactions has its own equilibrium constant so that the final expression for the percentage of hemoglobin contains four constants. Pauling (192) submitted a simplified expression which contained two constants. It is beyond the scope of this review to discuss these expressions in further detail.

According to the hypothesis of intermediate compound formation, carbon monoxide could also be conceived to react stepwise with each of the four ferrous atoms. Indeed, according to Lemberg and Legge (153), twenty-one classes of intermediates are possible in which the four hemes are free, or are combined with oxygen, carbon monoxide, or with both. In the case of the hemoglobin-methemoglobin equilibrium, Darling and Roughton (60) postulated the existence of the following intermediates: $(Fe^{++})_3\,(Fe^{+++})_1\,X$; $(Fe^{++})_2\,(Fe^{+++})_2\,X$; $(Fe^{++})_1\,(Fe^{+++})_3\,X$; $(Fe^{+++})_4\,X$. Apparently, the formation of the intermediate compounds as the result of combination of ferrous ion with carbon monoxide as in carboxyhemoglobin, or as the result of oxidation of ferrous to ferric ions as in methemohowever, the effect of methemoglobin is less than that of carboxyhemoglobin. For example, a content of 23 per cent carboxyhemoglobin shifted the dissociation curve of the residual oxyhemoglobin in human blood about as far to the left as did a content of 43 per cent methemoglobin. The effect of methemoglobin on the oxygen dissociation curve is wholly reversible. Eder and his associates (69) have recently reported that the position and shape of the dissociation curve are not altered in familial idiopathic methemoglobinemia.

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$$O_2 + (Fe^{++})(Fe^{+++})_3X \rightleftarrows (O_2)(Fe^{++})(Fe^{+++})_3X$$

IV. PHYSIOLOGY OF METHEMOGLOBINEMIA

In methemoglobinemia, as in carboxyhemoglobinemia, the decrease in hemoglobin available for oxygen transport together with the alteration in the shape and position of the dissociation curve constitutes a potential handicap to the organism in the unloading of oxygen in the tissues. We may now examine in somewhat greater detail the nature, extent, and consequences of this handicap.

A. Unloading of oxygen in the normal organism

Without entering into the vast details of the subject of respiration, we may mention briefly some of the salient features as a guide to our discussion of the effect of methemoglobinemia. The air in the alveolus of the lung contains oxygen at a tension or partial pressure of about 100 mm. Hg and carbon dioxide at a tension of about 40 mm. Hg. The alveolar air is practically in equilibrium with the arterial blood so that the tension of oxygen in the arterial blood is slightly lower and that of carbon dioxide slightly higher than in the alveolar air. Under normal conditions, the oxygen tension of the venous blood is about 40 mm. and that of carbon dioxide is about 45 mm. The oxygen tension in the tissues is less than 40 mm., perhaps even as low as 0 to 10 mm., depending upon the activity of the person. The carbon dioxide tension is higher than 46 mm. The arterial blood gives up oxygen to, and takes up carbon dioxide from, the peripheral tissues and it becomes venous blood in this process. The average oxygen dissociation curve for human blood, regardless of slight changes due to pH and CO_2 content, may be represented by figures, already given in Table V, which have been interpolated from a chart by Roughton and Darling (202). A change from an arterial oxygen tension of 100 mm. Hg to a venous oxygen tension of 40 mm. Hg implies a delivery of 19.4 minus 14.8 or 4.6 cc. of oxygen per 100 cc. of blood. This delivery occurs in a normal man at rest at sea level. A similar supply of oxygen can be furnished to the tissues under other conditions by virtue of the dissociability characteristics of oxyhemoglobin. For example, at high altitudes, as at an arterial oxygen pressure of only 50 mm. Hg, the delivery of 4.6 cc. of oxygen per 100 cc. of blood will be associated with a change to a venous tension of 30 mm. Hg. Again, in a normal man doing light work, the increased delivery of oxygen to the tissues may be reflected in a change from a normal arterial tension of 100 mm. of Hg to low venous tensions of 30 or even 20 mm. of Hg. In an anemia with a 40 per cent hemoglobin, a delivery of 4.6 cc.

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oxygen per 100 cc. of blood will be accompanied by a change from an arterial oxygen tension of 100 mm. to a venous oxygen tension of 22 mm.

B. Unloading of oxygen in the carboxyhemoglobinemic or methemoglobinemic organism

The delivery of oxygen to the tissues can be accomplished not only by way of the mechanism just described, namely, the dissociation of oxyhemoglobin but also by calling into play such cardiovascular mechanisms as increased cardiac output and increased blood flow through the tissues. The presence of carboxyhemoglobinemia or methemoglobinemia limits the extent to which oxygen can be supplied to the tissues as a result of the dissociation of the residual oxyhemoglobin. The question therefore arises concerning the exact degree of carboxyhemoglobinemia or methemoglobinemia at which the cardiovascular mechanisms are invoked.

Asmussen and Chiodi (7) found that induced carboxyhemoglobinemias of 20 to 33 per cent in men were not extensive enough to affect the cardiac output. For example, in one individual with a carboxyhemoglobinemia of about 33 per cent, the changes in the arterial-venous oxygen content and tension were as follows:

	REST	LIGHT WORK	HEAVY WORK
A-V oxygen content (vols. per cent)	12.1 − 7.3 = 4.8	12.7 − 4.6 = 8.1	13.0 − 2.8 = 10.2
A-V tension (mm. of Hg)	99 − 16	99 − 11	100 − 7

The cardiac output did not increase in any of these states of activity after the induction of carboxyhemoglobinemia had been induced. However, there were definite increases when the carboxyhemoglobin content approached or exceeded 40 per cent of the total blood pigments (44). For example, in one subject at rest, the cardiac output per minute was 3.8 liters at 0 per cent HbCO, 4.1 liters at 16 per cent, 5.8 at 32 per cent and 5.6 at 45 per cent.

Similar data for methemoglobinemia in man are not available. Clark and his associates (45) found that in dogs the respiratory ventilation was not increased no matter what the degree of methemoglobinemia. The cardiac output did not increase except at degrees of methemoglobinemia greater than about 40 per cent of the total blood pigment. In other words, when the methemoglobinemia is less than this level, the oxygen supply to the tissues is maintained by virtue of the dissociation characteristics of the residual hemoglobin. For example, their dog No. 1 without any methemoglobinemia showed an A-V oxygen difference of 18.0 minus 13.4 or 4.6 cc. per 100 cc. of blood. At a methemoglobinemia of 56 per cent, the A-V oxygen difference was 7.5 minus 4.0 or 3.5 cc. per 100 cc. of blood. Evidently, not enough oxygen could be supplied by dissociation of the residual oxyhemoglobin, and this was evidenced by an increase in the cardiac output from a level of 1.8 liters per minute before induction of methemoglobinemia to a level of 2.5 liters per minute at a level of 58 per cent methemoglobin.

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C. *Effect of methemoglobinemia on oxygenation of working muscle*

The data which we have just presented show that methemoglobinemia constitutes a potential impairment of the proper supply of oxygen to the tissues, first because less hemoglobin is available for oxygen transport and secondly because this residual hemoglobin has a greater affinity for oxygen and therefore unloads it less readily in the tissues. However, when the degree of methemoglobinemia is not excessive and the organism is at rest, an adequate oxygen supply is available. If the degree of methemoglobinemia exceeds about 40 per cent of the blood pigment, the cardiac output increases in order to aid in the supply of oxygen to the tissues.

Insufficient supply of oxygen to certain tissues results in a shift from the oxidative systems to the glycolytic systems in order to obtain the necessary energy (161). Such a shift is revealed by an increase in lactic acid production by the tissue with escape of this acid into the blood. The investigations of Bang (10) illustrate very forcibly this type of shift in man. During a prolonged period of mild work, the blood lactate rises at the beginning of the exercise. However, the cardiovascular and respiratory reflexes initiated by the exercise collaborate to deliver oxygen to the muscle at the rate required by the work; accordingly the liberation of energy switches back to the oxidative mechanisms and the blood lactate drops. If the work is strenuous, even though short, the oxygen supplied is not adequate; the muscle has to rely almost entirely on the anaerobic energy of glycolysis, and this is evidenced by a marked elevation of blood lactate and a slow return to normal levels. The extent of the rise in blood lactate after a measured amount of work may be used as a measure of the adequacy of oxygen delivery to the muscle tissue under non-resting conditions. The rate of delivery under these conditions may be inadequate at high altitudes, in physically untrained individuals or in other circumstances (229).

The extent to which methemoglobinemia interferes with the oxygenation of muscle in work was investigated by Tepperman, Bodansky and Jandorf (229). In four untrained subjects who were subjected to a work test on a cycle ergometer for three minutes at a load of about 4500 to 6000 ft. lbs. (621 to 828 kg. M.) per minute, the blood lactic acid curve was essentially the same before and after the induction of methemoglobinemias ranging from 7.5 to 16.8 per cent of the blood pigment. In two similarly untrained subjects, the blood lactate curve was higher and more prolonged in the presence of 21.7 and 27.1 per cent methemoglobin than in the absence of any methemoglobin. Trained subjects who worked at comparatively light work loads showed no upward displacement of the blood lactate curves with 7 to 17 per cent methemoglobinemia. However, at work loads higher than about 6500 ft. lbs. (897 kg. M.) per minute for three minutes, 10 to 20 per cent of methemoglobinemia resulted in impaired oxygenation of muscle as evidenced by elevated blood lactate curves.

D. *Effect of methemoglobinemia on visual threshold*

It has been noted by several groups of investigators that a rise in the threshold of the dark adapted eye occurs during anoxia induced by low oxygen tensions.

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McFarland and his associates (168) found that carboxyhemoglobinemia raises the brightness discrimination threshold in cone vision as measured by the Crozier and Holway visual discriminometer (59) and that this effect and the increase in threshold at low oxygen tensions were additive. Bodansky and Hendley (25) observed that there was no significant increase in the visual threshold in individuals having concentrations up to 30 per cent methemoglobinemia either at sea level or at simulated high altitudes of 12,000 feet or 3660 meters (13 per cent oxygen) or 18,000 feet or 5490 meters (10 per cent oxygen). Short bouts of exercise lowered the rod threshold immediately after exercise in methemoglobinemic individuals, but not in normal individuals. However, five to ten minutes after exercise the threshold rose above the normal threshold to the same extent in both methemoglobinemic and nonmethemoglobinemic individuals.

It is somewhat difficult to account for the fact that methemoglobinemia does not affect the rod threshold whereas carboxyhemoglobinemia raises it. As was pointed out earlier in this review, methemoglobinemia usually shifts the position and alters the shape of the oxygen dissociation curve of the residual oxyhemoglobin much less than an equivalent degree of carboxyhemoglobinemia. Therefore, for a given amount of oxygen unloading, a lower venous and tissue oxygen tension would be reached in the case of carboxyhemoglobinemia than in the case of an equivalent degree of methemoglobinemia. This may explain why dark adaptation may be more readily impaired in the former instance than in the latter.

The rise in the rod threshold both in methemoglobinemic and nonmethemoglobinemic individuals, five to ten minutes after exercise, may be explained in terms of the acidosis of the post-exercise period, since Wald and his associates (238) have shown that acidosis causes a rise in the rod threshold whereas alkalosis causes a decrease. It is difficult to explain the decrease in threshold in methemoglobinemic individuals immediately following exercise.

E. Relation between degree of methemoglobinemia and symptomatology

The question naturally arises concerning the levels of methemoglobinemia at which the reserve of the oxygen dissociation curve and the circulatory adjustments, combined, prove inadequate in supplying oxygen to the tissues, as judged by the development of grave symptoms and the occurrence of death. The possibility exists that some symptoms and death may be due in certain cases to effects other than those of methemoglobin production *per se*. Vandenbelt, Pfeiffer and their associates (231) found that oral administration of p-aminoacetophenone and p-aminopropiophenone to dogs resulted in ataxia at 60 per cent methemoglobin, salivation and prostration at 75 per cent and loss of consciousness at 85 per cent. The lethal level of methemoglobinemia was between 87 and 95 per cent. Bodansky and Gutmann (24) injected dogs intravenously with varying doses of p-aminopropiophenone in propylene glycol. Of eight animals which developed maximal concentrations of methemoglobin ranging up to 81 per cent, all survived and were normal during the subsequent period

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It may be readily appreciated that, at levels of 85 to 90 per cent methemoglobin, complete dissociation of the residual oxyhemoglobin would involve a fall of the venous oxygen tension to zero, yet would deliver to the tissues only 2 to 3 cc. of oxygen per 100 cc. of blood, an amount below the needs of the resting organism. It has already been pointed out that the cardiovascular mechanisms for increasing the oxygen supply to the tissues are called into play when the concentration of methemoglobin exceeds 40 per cent of the blood pigments. Hence it may be reasonably concluded that the combination of the reserve of the oxygen dissociation curve and the circulatory adjustments begin to prove inadequate in supplying oxygen to the tissues, as judged by the occurrence of ataxia, prostration and unconsciousness, when the methemoglobin concentration exceeds about 60 per cent. Failure of an oxygen supply necessary for sustaining life could be expected to result when the methemoglobin concentration reaches about 85 per cent. In this connection, it is interesting to note that the lethal level of carboxyhemoglobinemia is about 60 per cent of the total blood pigment (157). As has already been noted, this degree of carboxyhemoglobinemia causes as marked an alteration in the shape and position of the oxyhemoglobin dissociation curve as much higher concentrations of methemoglobin.

It should be stressed, on the other hand, that not all methemoglobin producing compounds are lethal by virtue of the methemoglobinemia which they induce. Lester and his associates (158) showed that doses of p-aminophenol and β-phenylhydroxylamine which produced maximal concentrations of methemoglobin were not sufficient to cause death. Thus, a maximum degree of methemoglobinemia, about 65 per cent of the total blood pigment, was produced in the rat by 5 mg. of β-phenylhydroxylamine per kg. Increasing the dose did not result in any increase in the concentration of methemoglobin, but death occurred at a dose of 35 mg. per kg. The amounts of methemoglobin formed by acetanilid in the cat are proportional to the concentration of total hemoglobin, but formation ceases at a residual concentration of hemoglobin that is adequate for supporting life.

F. Methemoglobinemia and hemolysis

A number of phenomena have been reported which indicate an association between methemoglobinemia and hemolysis or hemolytic anemia. The repeated administration of methemoglobin-producing drugs may cause a hemolytic anemia. For example, Van Loon and his associates (232) found that the chronic administration of acetanilid up to 36 mg. per kg. caused little or no abnormal hematological changes in dogs; larger doses produced a hemolytic anemia without any evidence of bone marrow depression. When the drug was discontinued, the blood picture reverted to normal. Acetophenetidin produced similar effects but was not as active as acetanilid.

The exact mechanism of the drug-induced hemolytic effect is not known. These drugs are not directly hemolytic. The possibility exists that they or their metabolic products may render the cells more sensitive to hemolysis. However, Jung (136) has shown that, as judged by hemolysis in NaCl solutions of varying concentration, methemoglobinemic erythrocytes have the same resistance as normal erythrocytes.

G. Effect of methemoglobinuria on renal function

Bing (20) reported that the intravenous infusion of crystalline methemoglobin into acidotic dogs was followed by a fall in the effective renal plasma flow, the glomerular filtration rate and the tubular reabsorptive capacity for glucose. This was evidenced by anuria and rise in blood urea nitrogen. The animals usually became moribund by the third day. The renal lesions comprised hydropic degeneration of the proximal convoluted tubules, cellular necrosis in the distal segment and plugging of the collecting tubules. No such functional or structural changes developed in non-acidotic dogs which were infused with methemoglobin or hemoglobin, or in acidotic animals infused with hemoglobin or myoglobin. On the other hand, Harrison and his coworkers (95) were able to show impairment of renal function as the result of intravenous injections of hemoglobin as well as methemoglobin. Injection of methemoglobin into acidotic dogs appeared to produce a greater degree of renal impairment as judged by nitrogen retention, but the critical factor in the severity of the renal impairment, in these various instances, appeared to be the rate of urine flow.

V. PHARMACOLOGY OF METHEMOGLOBINEMIA

It is abundantly evident from the considerations we have so far presented that any compound will produce methemoglobinemia if it is capable of oxidizing hemoglobin, or of being metabolized to a compound which can oxidize hemoglobin. There is also the possibility that certain substances may activate the oxidation of hemoglobin to methemoglobin or may inhibit the cellular mechanisms of the erythrocyte which constantly tend to reduce methemoglobin to hemoglobin. The degree of methemoglobinemia formed by any particular substance will, therefore, depend upon the interplay of a number of factors: the mode of administration, whether oral or parenteral; the conversion in the intestinal tract by bacterial or other agents; the rate of absorption from the intestinal tract if the mode of administration is oral; the pattern of metabolism into oxidizing and non-oxidizing compounds; the rate of excretion of its metabolites; the level of the methemoglobin-reducing mechanisms in the organism, a level which may vary with species, age, and other influences not yet elucidated.

A. Direct oxidants of hemoglobin

A number of substances are capable of oxidizing hemoglobin directly to methemoglobin. This capacity may be revealed by adding such substances *in vitro* directly to blood, suspensions of erythrocytes, hemolyzed blood, or solutions of hemoglobin. This group includes the nitrites, chlorate, hydrogen peroxide, alloxan, the quinones, and dyes of high oxidation-reduction potential.

1. *Nitrites.* Perhaps the best known of these direct oxidants is nitrite. The interaction of nitrite with hemoglobin has been studied extensively. The early studies of Barcroft and Müller (12) indicated that the molar ratio of nitrite utilized to methemoglobin formed was 2, that is, two molecules of nitrite interacted with one molecule of hemoglobin to form one molecule of methemoglobin. However, ratios of about 1.0 have been reported by Van Slyke and Vollmund (235) and by Meier (176). More recently, lower values of 0.5 to 0.7 have been obtained by Austin and Drabkin (9), by Darling and Roughton (60), and by Greenberg and his associates (88). These results indicate that one molecule of nitrite reacts with two molecules of hemoglobin to form two molecules of methemoglobin. The reaction depends upon a number of factors, such as concentration of nitrite, temperature and pH. Greenberg (88) has pointed out that in some of the earlier work the conditions may not have been such as to permit the reaction to go to completion. Side reactions involving the formation of nitrosohemoglobin and nitrosomethemoglobin may also take place (146, 173).

Quantitative determinations of the *in-vivo* production of methemoglobin by nitrites appear to have been made first by Haldane and his coworkers (90) in 1897. A hypodermic dose of 300–330 mg. per kg. led to death in about thirty minutes, with a methemoglobin concentration of 74 per cent of the total blood pigment in one mouse and 91 per cent in another. The symptoms of poisoning were, progressively, motor incoordination, prostration, coma and death. In a rabbit injected with 130 mg. per kg., the methemoglobin rose to a maximum of about 55 per cent within approximately one hour, and began to decline at one and one half hours after injection and decreased to about 5 per cent in three and one half hours. Exposure to amyl nitrite also caused death with methemoglobinemias of about 80 per cent. Sakurai (205) observed in somewhat greater detail the relationship between toxicity and methemoglobin concentration. He found that the LD_{100} in cats was 35 mg. per kg. Death at this dose occurred in from fifty-five minutes to one hour and fifty-two minutes after subcutaneous injection; the concentration of methemoglobin at death ranged from 88 to 100 per cent. The LD_{100} in rabbits was 170 mg. per kg. Death occurred in periods similar to that in cats, and the methemoglobin concentrations at death ranged from 86 to 94 per cent. The rate of formation in cats was very rapid. Following an intravenous injection of 30 mg. per kg., concentrations of 22 to 37 per cent were apparent at fifteen minutes, and concentrations of 33 to 52 per cent at thirty minutes. Maximum concentrations began to be reached at one hour and thirty minutes.

More systematic investigations on the relationship in cats between the dose of intravenously administered nitrite and the maximal degree of methemoglobin have been submitted by Issekutz (132). He found that the maximal concentration of methemoglobin formed was directly proportional to the dose. As will be pointed out later, for the aryl amino and nitro compounds, the dose-methemoglobin response curve is not, as for nitrite, a straight line but an S-shaped curve. Issekutz (132) found that the molecular ratio of the methemoglobin formed to the nitrite injected ranged from 0.7 to 1.4 and averaged 0.9. The significance and interpretation of molecular ratios of maximal methemoglobin concentration to dose of drug will be discussed more fully later. Issekutz also observed that the simultaneous injection of alcohol did not influence the formation of methemoglobin by nitrite.

Nitrites may be present in various preserved or cured meats. The original source of this nitrite is either a preserving solution which contains such nitrite or nitrate which has been added to the meat as the potassium salt and which is reduced in the course of time to nitrite either by bacterial organisms or by enzymes present in the meat. There exists a considerable literature concerning the detection and determination of nitrite in meat and meat products. Cases of poisoning due to the ingestion of such foods are reported from time to time. There is substantial evidence in some of the cases that the signs of toxicity or the occurrence of death is due largely to the methemoglobinemia induced by these nitrites. Thus, Schrader (212) has reported three deaths in young children from the presence of nitrite in meat-pickling salt or brine; cyanosis and gastroenteritis were noted in these cases. Bushoff (34) reported two instances of mass poisoning from the use of broth prepared from insufficiently clarified pickle-nitrite concentrate. Naider and Venkatrao (183) reported thirty cases of fatal nitrite poisoning in man. They estimated the lethal dose for man to be about 2 grams of sodium nitrite or about 2.6 grams of potassium nitrite. Severe cyanosis due to methemoglobin formation was reported by Barilari and Benedetto (13) from the ingestion of sausage containing sodium nitrite.

2. *Nitrates.* When nitrates are ingested, reduction to nitrite occurs in the intestinal tract, and absorption of the nitrite leads to methemoglobinemia. Three important sources of such methemoglobinemia in man are the administration of bismuth subnitrate to patients with gastrointestinal disorders, the use of ammonium nitrate as a diuretic and the inclusion of nitrate-containing well water in the milk formula of infants.

The first fatal case of methemoglobinemia due to the ingestion of bismuth subnitrate was reported by Bennecke and Hoffman (19) in 1906. Other cases of poisoning, some of them fatal, were subsequently reported by Böhme (26), Nowak and Gutig (189), Roe (201), R. C. Miller (180), G. E. Miller (179), Wallace (230), and more recently by Marcus and Joffe (172). In the older cases bismuth subnitrate had been ingested as a consequence of its use as a radiopaque medium in roentgenological technics. More recent cases were due to the use of this compound in order to control diarrhea, particularly in infants. Marcus and Joffe's (172) case was in an infant who had received 0.1 gram of bismuth subnitrate every three to four hours for two days.

The use of ammonium nitrate as a diuretic has also resulted in the production of methemoglobinemia. In 1929, Eusterman and Keith (72) reported two nonfatal cases in adults. Tarr (226) later reported four new cases, and noted the existence of five others in the literature.

Of recent and considerable interest is the observation that young infants on milk formulas made with well water may develop methemoglobinemia. A number of such cases have been reported in this country within the past few years by Schwartz and Rector (215), Comly (50), Faucett and Miller (74), Chapin (42) and Stafford (224). Additional cases have been reported by Ferrant (75) from Belgium and by Medovy and his associates (174) from Canada. The etiology of this condition was established by Comly who found that well water used to make the milk formula in one of his cases contained 140 mg. nitrate nitrogen or 619 mg. of nitrate ion per liter. Chapin (42) found a concentration of 244 mg. nitrate nitrogen per liter. Medovy and his associates (174) reported a concentration of 250 mg. of nitrate per liter of well water. The nitrate ion comes, of course, from seepage of wastes into poorly constructed or located wells (243).

It has been established that the intestinal flora includes organisms which are capable of converting nitrate to nitrite ion. Apparently, if sufficient nitrate ion is introduced, this conversion is extensive enough so that large amounts of nitrite ion are absorbed into the blood stream. The subsequent oxidation of hemoglobin to methemoglobin has already been discussed in detail.

3. *Chlorates.* The capacity of potassium chlorate to form methemoglobin formerly attracted considerable attention because of the use of this compound in mouth-washes, gargles and dentifrices. When potassium chlorate is added to blood *in vitro*, methemoglobin formation is very slow or negligible at first but accelerates subsequently. Thus Richardson (200) found that the addition of potassium chlorate to human blood in a final concentration of 0.25 per cent did not lead to any methemoglobin formation in eight hours, but that complete conversion with some hemolysis occurred in twenty-four hours. When the blood was hemolyzed before the addition of chlorate, definite methemoglobin formation occurred in eight hours.

The *in-vivo* effects of potassium chlorate were first investigated by Marchand (171) in 1887; he observed that dogs given oral doses of about 1.8 to 2.7 gram of $KClO_3$ per kg. body weight showed methemoglobin formation in the blood. Such formation was not unequivocally demonstrated at lower doses. However, a number of other investigators were not able to demonstrate methemoglobin formation except after death. Richardson (200) did not find any significant methemoglobin formation in pigeons which received an average total oral dose of 26 grams in their drinking water over periods of thirteen to fifty-three days, even though death occurred. Average total intramuscular doses of 13.5 grams similarly failed to produce any methemoglobin formation. On the other hand, potassium chlorate was capable of forming methemoglobin in cats, provided that the dose was large enough. Thus, of three cats receiving 0.5 gram of $KClO_3$ per kg., two showed some methemoglobin formation although all three cats died.

The mechanism of methemoglobin formation by chlorate has been more recently investigated by Jung and his associates (113, 134) who found that repeated subcutaneous doses of 1 Gm. per kg. produced no methemoglobin in rats whereas one such dose led to high concentrations of methemoglobin in cats, with death at levels ranging from about 50 to 80 per cent. Of interest was the finding that no methemoglobin was formed for two hours following the administration of potassium chlorate, and the rate then followed an S-shaped or autocatalytic type of curve. The existence of the latent period had been previously observed by Littardi and Zanichelli (164) and corresponds to that observed *in vitro* by Richardson (200). Becker and his coworkers (18) also noted that in rats, although chlorate ion produced no methemoglobinemia, the conjoint use of small doses of sodium nitrite and chlorate led to much higher degrees of methemoglobinemia than could be produced by nitrite alone. The use of methemoglobin-binding compounds also decreased the methemoglobin-forming power and toxicity of chlorates in the cat. These various observations have led to the formulation that the methemoglobin-forming action of chlorate *in vivo* is an autocatalytic phenomenon.

4. *Quinones.* That quinones can form methemoglobin *in vitro* has recently been demonstrated by Cannava and his associates (36–40), and by Hoffman-Ostenhof and his coworkers (121, 122). Thus the latter showed that a series of benzoquinone and naphthoquinone derivatives, including benzoquinone itself, 2-6-dichloroquinone, toluquinone and 1-2-napthoquinone, converted hemoglobin to methemoglobin directly in hemolyzed and nonhemolyzed dog blood at pH 6.8. However, this oxidation was not dependent upon the oxidation-reduction potential of the various quinones.

Cannava (39) found that the minimal dose of 2,3-dimethyl-1,4-napthoquinone necessary to produce methemoglobin in dogs was 2.5 mg. per kg. A dose of 20 mg. per kg. led to the development of about 30 per cent methemoglobin in twelve to twenty-four hours with a gradual diminution in two to four days. Similar degrees of formation of methemoglobin were found after the administration of 2-methyl-1,4-napthoquinone and of 2-methyl-1,4-napthohydroquinone (38).

An interesting insight into a possible physiopathological role of quinones in methemoglobin formation has been submitted by Fishberg (77, 78) who noted that urine in pathologic states brought about by ascorbic acid deficiency was capable of producing methemoglobin *in vitro*. It was found that a patient with "autotoxic enterogenous cyanosis" excreted large amounts of benzoquinone acetic acid, that this excretion could be suppressed by the administration of ascorbic acid, and that the amount of methemoglobin in the blood varied with the urinary excretion of benzoquinone acetic acid and hence with its formation as the result of a deranged metabolism of tyrosine.

5. *Other direct oxidants.* There are a number of other substances which convert hemoglobin to methemoglobin when added directly to intact or hemolyzed red cells or to hemoglobin itself. We have already considered the mechanisms which are involved in this conversion, and we shall now submit some data concerning the extent of methemoglobin formation *in vivo*.

Combemale (49) in 1891 showed that a dose of 500 mg. of methylene blue per kg. induced methemoglobinemia in dogs. Nadler and his associates (182) found that the intravenous injection of 500 mg. of methylene blue in man or a dose of about 7 to 10 mg. per kg. produced methemoglobin concentrations of about 0.4 to 8.3 per cent, as determined manometrically. Bock (21) reported that lethal or near-lethal subcutaneous doses of 75 mg. per kg. in the dog and 100 mg. per kg. in the cat and the mouse produced methemoglobin concentrations of approximately 10 to 15 per cent about an hour after injection. The concentration of methemoglobin then decreased and subsequently, usually in the course of several days, rose to higher levels, about 30 to 50 per cent methemoglobin. As we shall note later, methylene blue is extremely effective in reducing methemoglobinemia. This apparently paradoxical capacity, namely, the capacity to produce methemoglobin in the normal organism and to cause its disappearance when it is already present in large amounts is discussed more fully in the section on Treatment.

We have already considered the role of hydrogen peroxide in the formation of methemoglobin. In the course of studies on the parenteral administration of hydrogen peroxide, Lorincz and associates (166) observed that methemoglobin was formed in those species with very low blood levels of catalase.

B. *Indirect oxidants of hemoglobin*

There is a large group of aromatic amino and nitro compounds which, as a whole, either do not form methemoglobin from hemoglobin *in vitro* or do so only very poorly. Yet these compounds are for the most part very active formers of methemoglobin *in vivo*. The possibility therefore naturally arises that such compounds are metabolized into some extremely active intermediate compounds.

It is manifestly burdensome to describe in detail the vast amount of work which has been done on the methemoglobin-forming capacity of these various aromatic amino and nitrogen compounds. In the hands of different investigators, the conditions of *in-vitro* experiments have varied greatly. Similarly, in *in-vivo* studies, there have been differences in the species of animal employed, the dose of the compound, the medium in which the compound was dissolved and the route by which it was administered, the times at which blood samples were taken for methemoglobin determination, and the precision of the methods by means of which the methemoglobin was determined. It appears preferable to review rather that work which illustrates certain generalizations which may be made concerning the methemoglobin-forming capacities of these compounds.

1. *In-vitro formation of methemoglobin by aryl amino and nitro compounds.* In order to evaluate the possibility that various aromatic amino and nitro compounds may exert their *in-vivo* action by being converted into compounds like aminophenols or hydroxylamines which are direct oxidants of hemoglobin, it is necessary to consider the available data on the *in-vitro* action of these compounds.

In 1913, Heubner (112) noted that the direct addition of the aminophenols to whole blood led to formation of methemoglobin; the order of effectiveness of the different isomers was: ortho, para, meta. The addition of 0.25 mg. ortho-aminophenol to 2 cc. of hog's blood, equivalent to 12.5 mg. per 100 cc., produced a marked methemoglobin band in the spectrum in twenty-four minutes. Since the proportions in this conversion are about 0.1 mM of *o*-aminophenol and 1 mM of hemoglobin, Heubner calculated that 1 molecule of the former compound was capable of converting 10 molecules of hemoglobin. However, no methemoglobin was formed in the absence of atmospheric oxygen. Heubner also noted that aniline and metaxylidine but not dimethylaniline were capable of forming methemoglobin *in vitro*. These observations, as we shall later note, led Heubner to conclude that the aminophenols were a necessary intermediate in the formation of methemoglobin *in vivo*.

The hydroxylamines are also active formers of methemoglobin *in vitro*. Heubner and his associates (115) noted marked methemoglobin formation five minutes after mixing of 0.006 per cent of hydroxylamine with diluted cattle blood, and calculated that about 0.6 to 1 molecule of hydroxylamine was capable of converting 1 molecule of hemoglobin to methemoglobin. Lipschitz (163) also noted that hydroxylamine readily formed methemoglobin *in vitro*; the action of nitrophenylhydroxylamine was much weaker. Ellinger (71) observed that incubation of acetanilid with defibrinated cat's blood led to a gradual formation of methemoglobin as measured by reduction in the oxygen capacity; this amounted to about 35 per cent in the course of about forty-eight hours. However, no such decrease occurred with oxalated or citrated blood. As is well known, acetanilid forms methemoglobin *in vivo*. No methemoglobin was formed with acetyl-n-methylaniline or acetyl-p-toluidine either *in vivo* or *in vitro*. These observations led Ellinger (71) to conclude that acetanilid exerted its methemoglobin-forming action by being converted to acetylphenylhydroxylamine.

2. *Effect of animal species.* It was repeatedly noted in the older literature that different species of animals showed varying susceptibilities to methemoglobin formation by a stated compound. More recently, systematic quantitative studies have been made by Lester (156) and Spicer (220). The former worker studied the maximal amount of methemoglobin formed by acetanilid and acetophenetidin at various doses, expressed as millimols per kg. Using as a criterion of sensitivity the reciprocal of the dose required to produce a stated concentration of methemoglobin, Lester (156) found that the cat was most sensitive. If this species is listed as 100, the sensitivities of the other species are as follows for acetanilid: man, 56; dog, 29; rat, 5; rabbit, 0; monkey, 0. For acetophenetidin, the sensitivities were as follows: cat, 100; man, 63; dog, 35; rat, 5.

There is a considerable number of other studies in the literature which, although not as systematic as those of Lester, show the extreme sensitivity of the cat to methemoglobin formation by other compounds. For example, Reiter (199) found that in guinea pigs, a dose of 300 mg. of p-nitro-o-toluidine per kg. of body weight never produced more than 1 gram of methemoglobin per 100 cc. or about 15 per cent of the total blood pigment. In contrast, in the cat much smaller doses produced considerably higher degrees of methemoglobinemia.

Thus, 1 mg. per kg. yielded 1.3 gram of methemoglobin per 100 cc. or 12 per cent of the total blood pigment; 10 mg. per kg. yielded 5.9 grams or 56 per cent of the total pigment. Jung (138) found that about twice as much nitrosobenzol or phenylhydroxylamine was required in the rat as in the cat to yield similar ranges of maximal methemoglobin concentrations. According to Spicer (220), 15 mg. of aniline per kg. produced 28.3 per cent of methemoglobin in the dog, 56.4 per cent in the cat, and negligible amounts in the rat. The cat has been used almost entirely for the evaluation of methemoglobin-forming compounds because of the ease with which this pigment is formed in this species.

3. *Methemoglobin-forming capacity of aryl amino and nitro compounds.* Since aryl amino and nitro compounds do not readily oxidize hemoglobin *in vitro*, it is apparent that the formation of methemoglobin *in vivo* must be accomplished through some metabolic derivative. It has been held that such a derivative either oxidizes hemoglobin directly or interacts with oxyhemoglobin to form hydrogen peroxide which then oxidizes hemoglobin (153). We shall presently discuss these alternative hypotheses in detail. It is important to consider first, in quantitative terms, the methemoglobin-forming capacities of these various compounds.

As already pointed out in this paper, when a methemoglobin-forming compound is administered, methemoglobin begins to accumulate in the blood at a rate characteristic of the compound, attains a maximum concentration at which it remains for a variable period of time and then begins to decrease. The specific shape of the curve is the resultant chiefly of three processes: (a) metabolism of the compound to other substances which do not oxidize hemoglobin and which, with respect to our present interest, may be regarded as side reactions; (b) formation of an intermediate compound which reacts directly with hemoglobin to form methemoglobin; (c) back reduction of methemoglobin, as it is being formed, to hemoglobin by the enzyme systems of the blood. These three processes are continuous and interdependent. Although the kinetics of the back reduction of methemoglobin has received some study and, in general, appears to be independent of the particular methemoglobin-forming compound, the kinetics of the other two processes have only been very fragmentarily explored, and then only for a few compounds. It is therefore impossible at the present time to characterize with any precision the methemoglobin-forming capacity of various compounds by any general equation.

It may, however, be postulated that the maximum concentration of methemoglobin formed is dependent upon the total number of electrons accepted from hemoglobin by an intermediate oxidation agent, and that the concentration of the intermediate oxidation agent is proportional to the dose of the aryl amino or nitro compound administered to an animal. These assumptions would lead to an equation of the form, $\frac{[\mathrm{Hb}]\,[\mathrm{D}]}{[\mathrm{MetHb}]} = K$, where [Hb] and [MetHb] are the concentrations of hemoglobin and methemoglobin, respectively, per 100 cc. of blood or per kg. of body weight, where D is the dose per kg. of body weight and where K is the methemoglobin-forming capacity of the particular com-

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It is, however, of little value to attempt the application of a Hüfner-like equation or of more refined expressions to the data which are at present available in the literature. The value for the maximal concentration of methemoglobin at a stated dose may vary considerably in individuals of the same species; Bredow and Yung's (28) data for a dose of 70 mg. per kg. in the cat show the following values for four individuals in mM of methemoglobin per kg.: 0.066, 0.22, 0.30, 0.36. Such variability is probably an expression of the differing capacities of the individuals of a species in the metabolism of the drug or in the enzyme systems involved in the back reduction of methemoglobin. However, it is quite apparent that a dose-methemoglobin curve based, as it has been, on the use of one individual for each dose must inherently be subject to great error.

A review of the literature on the methemoglobin-forming capacity of various compounds shows that this has been formulated most frequently as the average molecular ratio of the methemoglobin formed to the dose of the compound; both quantities are expressed per kg. body weight. The cat has been the species most frequently used. The molecular value for the methemoglobin is obtained by multiplying the concentration in the blood, expressed as grams per 100 cc., by the value, 0.75 or 0.70, which represents the blood volume per kg. body weight. The values for the molecular ratio at different doses are then averaged, even though the individual values may differ greatly from each other.

We have already described the general nature of the relationship between doses of a methemoglobin-forming compound and the concentration of methemoglobin. It is apparent that the molecular ratio will decrease at high dosages. For example, Reiter's (199) data on p-nitro-o-toluidine show molecular ratios decreasing from 9.2 at a dose of 1 mg. per kg. to 1.6 at a dose of 30 mg. per kg.; averaging of these values for the molecular ratios at the different dosages can only yield a crude representation of the methemoglobin-forming capacity. Thus, Reiter (199) gives an average molecular ratio of 3.7. The standard error of this mean value is ±0.78. In other words, this mean value of 3.7 cannot be said to differ significantly from molecular ratio values greater than about 1.5 or less than 5.9 and which have a similar standard error.

The possibility arises that a more precise estimate of the methemoglobin-forming capacity may be obtained if only a limited portion of the dose-methemoglobin concentration curve is employed for the calculation of the molecular ratios. Theoretical considerations as well as direct examination of data in the literature would appear to indicate that the dose-methemoglobin curve does pound. Such an equation is of the same mathematical form as the Hüfner (130) equation for the hemoglobin-oxyhemoglobin equilibrium. Indeed, the data on certain compounds such as that of Reiter (199) on nitro-o-toluidine or of Petersen (196) on nitrosobenzol are sufficiently extensive to show that the concentration of methemoglobin increases with increasing doses of the compound until a point is reached beyond which relatively large increases in the dose of the drug produce small or negligible increases in the concentration of methemoglobin.

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It is apparent, therefore, that the method which has been used so widely for calculating the average molecular ratio has not in general yielded precise values for methemoglobin-forming capacity. Nor can such data usually be treated or employed to yield more precise values. It is, of course, possible that the use of greater numbers of animals of a given species or the use of a more uniform strain of a given species may yield averages with less variance. For purposes of record, we list in Table VI the average molecular ratios which have been obtained by different investigators.

Although we cannot place too much quantitative emphasis on these results, the above tabulation reveals two findings which are qualitatively impressive. It may be noted, first, that the molecular ratio is greater than one for a considerable number of compounds. In other words, one molecule of each of these compounds forms an intermediate which can accept more than one electron. Since hemoglobin yields one electron when it is converted to methemoglobin and since probably only a small fraction of the original compound is metabolized to an oxidizing intermediary compound, then this intermediary compound must partake in a turnover reaction, being alternately reduced as it reacts with hemoglobin and converted by other systems back to an oxidizing intermediate.

The second point of interest in Table VI is that p-dinitrobenzene and phenylhydroxylamine are oustanding methemoglobin formers. Ortho-aminophenol, m-dinitrobenzene and nitrosobenzene, though not as distinctive in this respect, still appear to be much more active than the other compounds. In 1944, Vandenbelt, Pfeiffer and their associates (231) reported that p-aminoacetophenone and p-aminopropiophenone were extremely potent methemoglobin formers. Unfortunately, their determinations were done on dogs and do not permit direct comparison with other compounds for which the data has been obtained in cats. However, the data of Vandenbelt and his associates (231) and of Bodansky and Gutmann (24) permit calculation of a molecular ratio of about 30 for p-aminopropiophenone and of about 10 for p-aminoacetophenone when these agents are intravenously administered. If it can be assumed that the cat and the dog have the same relative sensitivities to these compounds as they have been shown to have to acetanilid and acetophenetidin, then the molecular ratios for p-aminopropiophenone and p-aminoacetophenone in the cat would be about 100 and 30, respectively, and therefore of about the same order of magnitude as that of m-dinitrobenzene.

The finding that certain compounds are potent methemoglobin formers naturally raises the possibility that these compounds or compounds easily derived from them possess a chemical grouping which, either directly or in association with other systems, is of critical importance in the oxidation of hemoglobin. Thus, because of their relatively high methemoglobin-forming capacity, p-aminophenol, phenylhydroxylamine and nitrosobenzene have been implicated by various investigators as being the important intermediates in the metabolism of other methemoglobin-forming compounds. We shall now consider the extent to which these implications are substantiated by data on the intermediary metabolism of various methemoglobin-forming compounds.

TABLE VI

Methemoglobin-forming capacity of aryl amino and nitro compounds in the cat

COMPOUND	MOLECULAR RATIO	REFERENCE
Aniline	2.5	Herken (110)
	2.7	Schwedtke (216)
Acetanilid	1.0+	Lester (156)
m-phenylenediamine	1.4	Jung (137)
Acetophenetidin	0.14+	Lester (156)
o-Aminophenol	6.8+	Petersen (196)
Nitrosobenzene	8.6+	Petersen (196)
p-Aminophenol	3.6	Schwedkte and Sing (217)
p-Aminophenol	1.3	Issekutz (122)
Phenylhydroxylamine	34.0	Issekutz (122)
Nitrobenzene	0.86	Bredow and Jung (28)
o-Dinitrobenzene	1.9	Heubner and Sing (118)
	3.7	Issekutz (122)
m-Dinitrobenzene	7.1	Issekutz (122)
	7.8	Bredow and Yung (28)
	6.4	Heubner and Sing (118)
p-Dinitrobenzene	55	Heubner and Sing (118)
	198	Issekutz (122)
Trinitrobenzene	4.8	Bredow and Jung (28)
o-Nitrotoluol	0.05	Bredow and Jung (28)
m-Nitrotoluol	0.04	Bredow and Jung (28)
p-Nitrotoluol	very slight	Bredow and Jung (28)
2,4-Dinitrotoluol	1.4	Bredow and Jung (28)
2,6-Dinitrotoluol	0.55	Bredow and Jung (28)
2,4,6-Trinitrotoluol	1.7	Bredow and Jung (28)
m-Chloronitrobenzene	2.3	Jung (137)
m-Aminonitrobenzene	3.0	Jung (137)
2,4-Dinitrochlorbenzene	0.6	Jung (137)
p-Nitro-*o*-toluidine	3.7	Reiter (199)

4. *Sulfonamides.* The introduction of sulfonamide drugs into medical practice about 1937 gave rise to the hazard of methemoglobinemia. The first cases were reported by Gibberd (81) and by Paton and Eaton (190). Hartmann and his associates (96) found that, in the great majority of patients receiving 0.1 gram or more daily of sulfanilamide per kg. of body weight, some cyanosis due to

methemoglobinemia was detectable. In general, there was an approximate correlation between the sulfanilamide concentration in the blood and the methemoglobin concentration; levels of 10 to 20 mg. of sulfanilamide per 100 cc. were usually associated with methemoglobinemia ranging from 20 to 30 per cent. Several other groups at about this time reported a high incidence of methemoglobinemia in patients receiving sulfonamides (79, 94, 237). With the replacement of sulfonamides by antibiotics in the treatment of various bacterial infections, the reported incidence has decreased greatly.

We shall presently discuss the intermediate metabolism of methemoglobin-forming compounds and the probable mechanisms and note that the formation of methemoglobin is due to the oxidizing action of some intermediate, the precise nature of which is not yet established. There appears to be some question whether a similar intermediate is involved in the action of sulfanilamide. Harris and Michel (94) and Fox and Cline (79) were inclined to the view that the action was indeed due to the oxidizing compounds formed in the metabolism of sulfanilamide. On the other hand, there has been some evidence that sulfanilamide inhibits the action of catalase. Such a finding would fit in with the theory that methemoglobin formation is due to the action of hydrogen peroxide (48).

In connection with cyanosis due to sulfanilamide administration, it has been pointed out by several investigators that such cyanosis may in part be due to colored intermediates. Indeed, Kallner (141) considers that the cyanosis is largely due to the combination of carbhemoglobin with sulfanilamide.

5. *Intermediary metabolism of methemoglobin-forming compounds and the oxidation of hemoglobin.* The metabolic fate of aniline derivatives has claimed much attention. In 1913, Heubner (112) observed that the direct *in-vitro* addition of various aminophenols to whole blood caused the formation of methemoglobin, in the proportion of several molecules of the latter per molecule of compound. He also observed that p-aminophenol, when allowed to stand in air, was readily converted to quinone imine. Heubner assumed, therefore, that aniline and its derivatives were metabolized to p-aminophenol which was then oxidized to quinone imine. The latter then oxidized hemoglobin to methemoglobin, being itself reduced back to p-aminophenol. The cycle could be repeated a number of times (112, 116).

Greenberg and Lester (86, 87), however, have recently submitted data which do not support Heubner's formulation. Heubner observed methemoglobin qualitatively on spectroscopic examination of cow's blood to which p-aminophenol in concentrations of at least 25 mg. per 100 cc. had been added. Greenberg and Lester found that no methemoglobin was formed *in vitro* at concentrations of 0.1 and 0.5 mg. of p-aminophenol per 100 cc., and occasionally and only to a negligible degree at 1.0 mg. per cc. Definite methemoglobin formation resulted at much higher concentrations, namely, 13.2 per cent methemoglobin at 5.0 mg. of p-aminophenol per 100 cc., and 21.2 per cent at 10 mg. of p-aminophenol per 100 cc. But Greenberg and Lester (87) found that methemoglobinemia could be produced in man without the appearances of any appreciable concentration of p-aminophenol in the blood. Thus, a human subject who received

0.975 gram of acetanilid showed a concentration of 4 per cent methemoglobin an hour after administration and 8 per cent methemoglobin at two hours; yet the concentration of p-aminophenol was less than 0.005 mg. per 100 cc., far below the amount required to produce methemoglobin *in vitro*. Moreover, the administration of 500 mg. of p-aminophenol resulted in the appearance of unconjugated p-aminophenol in the blood one half and one hour later; yet no methemoglobin was found. Thus, these experiments show that there is no necessary association between the presence of p-aminophenol and methemoglobin formation. It was also observed that the major metabolites of acetanilid, namely, N-acetyl p-aminophenol and its hydroxy conjugates with sulfuric and glycuronic acid, are not capable of forming methemoglobin either *in vivo* or *in vitro*.

It will be recalled that phenylhydroxylamine is also a potent methemoglobin former (132, 207). In 1920, Lipschitz (163) assumed that this compound was the essential intermediary step in the action of aniline and its derivatives. He considered that free aniline was formed and was then oxidized to phenylhydroxylamine and that the latter compound oxidized hemoglobin to methemoglobin, being itself reduced to aniline. Thus, there was set up a cyclic oxidation-reduction reaction in which several molecules of hemoglobin could be oxidized to methemoglobin by one molecule of aniline.

The existence of the oxidation-reduction reaction, phenylhydroxylamine + hemoglobin $\rightleftarrows$ nitrosobenzene + methemoglobin has, however, been disputed. Heubner and his associates (116) were unable to find that phenylhydroxylamine could oxidize reduced hemoglobin *in vitro*. Jung (138), however, observed that phenylhydroxylamine could be oxidized to nitrosobenzene by oxyhemoglobin, and that the nitrosobenzene in turn oxidized *reduced* hemoglobin to methemoglobin. Other formulations of the intermediary metabolism of acetanilid have been recently summarized by Greenberg and Lester (87).

The presence of phenylhydroxylamine or nitrosobenzene in blood after administration of acetanilid has not yet been established. Ellinger (71) isolated a small amount of N-acetyl-phenylhydroxylamine from the blood of cats given acetanilid. Jung (138) pointed out that if phenylhydroxylamine and nitrosobenzene were both present in the body they might condense to form azoxybenzene, and this compound might be transformed into other azo compounds. Greenberg and Lester (87) found that the amount of azo compounds appearing in the urine after administration of acetanilid corresponded to about 0.5 per cent of this compound.

The intermediary metabolism of the other potent methemoglobin formers, namely, p-dinitrobenzene, p-aminoacetophenone, and p-aminoacetopropiophenone, has not been investigated, and it is difficult, therefore, to venture any opinion whether intermediates through which they pass are similar to those postulated in the metabolism of acetanilid.

There is at present, therefore, no conclusive evidence concerning the nature of the intermediary compound which oxidizes hemoglobin to methemoglobin. That it is not an immediate derivative of p-aminophenol appears probable from the work of Greenberg and Lester (87). However, the intermediate may be present in such small amounts that it has until now eluded chemical detection or analysis. It may be stressed that the minuteness of the quantity in which a metabolite of a methemoglobin former may be found to be present in the blood or urine does not militate against its role as an essential intermediate in the oxidation of hemoglobin to methemoglobin, for it is possible that such an intermediate may have a high turnover number and partake many times in a cyclic oxidation-reduction reaction.

The recent report of Spicer and Neal (222) is of interest in demonstrating the role of oxygen in the *in-vivo* formation of methemoglobin by an aryl amino compound, aniline. These investigators found that dogs exposed to simulated high altitudes (28,000 feet or 8540 meters) formed less methemoglobin in response to a given dose of aniline, 15 mg. per kg., than when they were exposed to normal atmospheric oxygen tension. There was, however, no difference in the response of methemoglobin formation to nitrite. It would appear, therefore, that low oxygen tension affects either the conversion of aniline to an oxidizing intermediate or the process of oxidation of hemoglobin to methemoglobin. The effect does not appear to be on the conversion of aniline to p-aminophenol or nitrosobenzene, for it was found that these latter substances also led to greater formation of methemoglobin at normal than at low tensions of oxygen.

In view of these results and those obtained on the intermediary metabolism of acetanilid, it would appear of value to investigate the mechanism of oxidation of hemoglobin to methemoglobin by aryl amino and nitro compounds in less complicated systems than the whole organism, such as in tissue slices, homogenates or extracts.

6. *Industrial hazards.* Various industrial processes involve exposure to methemoglobin-producing compounds. Thus, in electric arc welding, nitrous oxides are generated; as a rule, the results of such exposure are negligible for the concentration of methemoglobin is seldom more than 3 per cent (167). Although TNT is a methemoglobin producer, the effect of ingestion of small amounts, about 1 mg./kg., is negligible in man and there is no detectable change in the blood picture (129). A few years ago, Bass and his coworkers (17) reported the occurrence of methemoglobinemia in men who washed assembled roller bearings in mineral seal oil. This oil was found to contain 0.5 per cent of a methemoglobin former, 2-anilino-ethanol.

VI. TREATMENT OF METHEMOGLOBINEMIA

A. *Symptomatology of methemoglobinemia in man*

Except for cyanosis of the mucous membranes, dogs do not show any symptoms due to methemoglobinemia at concentrations of methemoglobin less than 60 per cent. The predominant symptoms are salivation, ataxia and vomiting at levels of 60 to 70 per cent, and ataxia and vomiting at levels of 71 to 80 per cent. At concentrations of 81 to 90 per cent, loss of consciousness is the outstanding feature. Lack of determinations of methemoglobin concentrations in the clinical literature make a similar correlation in man somewhat difficult.

Cyanosis does not become apparent until the concentration of methemoglobin reaches a level of about 15 per cent of the total pigment (23). Of course, the higher the concentration of methemoglobin the more striking is the cyanosis. Methemoglobinemias up to 30 per cent do not in general produce any symptoms, although occasionally dyspnea occurs upon exertion (76). At levels from 30 to 45 per cent, exertional dyspnea may be present if the methemoglobinemia has been rather acutely induced by the ingestion of various drugs. On the other hand, in familial idiopathic methemoglobinemia, which is usually present from birth, symptoms of fatigue, exertional headaches and reduced exercise tolerance may be mild or absent (76). This absence of symptoms may be explained by the secondary polycythemia compensating for inert pigment and by the lack of any shift in the position of the oxyhemoglobin dissociation curve (69). From the few available reports, it would seem that lethargy and semi-stupor do not become evident until levels of about 60 per cent are attained. The recorded existence of such levels without unconsciousness would seem to indicate, by analogy with the symptoms in animals, that the lethal level may well be above 70 per cent and approach the lethal level in dogs, namely, about 85 per cent (24).

B. *Moderate drug-induced methemoglobinemias*

It has been previously indicated that, following the administration of methemoglobin-forming drugs, the concentration of methemoglobin rises at a rate characteristic of the compound until it attains a maximum, persists there for some time, and then decreases again to negligible levels. Most cases of methemoglobinemia which are induced by the administration of a drug or other compound and in which the concentration does not exceed about 40 per cent of the total blood pigment can therefore be treated by "watchful waiting," provided the patient does not engage in strenuous physical activity which may tax his oxygen-unloading mechanisms. A knowledge of the particular compound which has been administered or inadvertently taken will aid in prognosis. For example, if one may judge from the results obtained in dogs, the methemoglobinemia due to nitrite may reach a maximum in about an hour and vanish within a few hours while that due to nitrobenzene may not produce its maximum effect for fifteen hours and then recede only slowly (58). There are few data on the rates of development and disappearance of methemoglobinemia induced by different drugs. Hartmann, Perley and Barnett (96) noted that the methemoglobinemia due to sulfanilamide administration decreased from a 20 per cent to zero level within twenty to seventy hours after withdrawal of the drug. The author observed that methemoglobinemias of 20 to 30 per cent due to the administration of a single oral dose of p-aminopropiophenone had decreased practically to zero level within twenty-four hours (23).

The treatment in such cases may consist simply in discontinuation of the drug, with an assurance to the patient that the methemoglobinemia will recede spontaneously within a day or two. However, the associated cyanosis may produce great anxiety in the patient or his family or may be disagreeable for cosmetic reasons. In cases where these conditions hold, it is advisable to institute more active treatment.

C. *Familial idiopathic methemoglobinemia*

As has already been pointed out in this review, familial iodiopathic or congenital methemoglobinemia is a condition in which cyanosis is present at birth to varying extents and which may become aggravated in childhood or early adulthood. Recent investigations have established that the cyanosis is due to methemoglobinemia as the result of derangement in the enzyme systems which are involved in the maintenance of the hemoglobin-methemoglobin equilibrium of the erythrocyte. We have previously considered this aspect in some detail. The outstanding symptom is breathlessness upon exertion. Secondary polycythemia develops. In 1948, Finch (76) noted that sixteen cases of the disease had been reported in the literature. In general, the concentrations in these cases range from about 30 to 45 per cent of the total blood pigment.

The two substances which have found the most widespread use in the treatment of congenital as well as other types of methemoglobinemia are methylene blue and ascorbic acid. Possibly because of its oxidation-reduction properties, methylene blue was regarded about twenty years ago as an antidote in the treatment of types of poisoning, such as those due to cyanide, carbon monoxide or methemoglobin formation, in which, through one way or another, cellular uptake of oxygen was denied (92). It is beyond the scope of this paper to consider the merits of this assumption insofar as carbon monoxide or cyanide poisoning is concerned. Hauschild (101–103) first demonstrated in animals that the toxicity of methemoglobin-forming compounds like sodium nitrite and phenylhydroxylamine could be counteracted by the administration of thionine or methylene blue and that these compounds could reduce methemoglobin to hemoglobin *in vivo* and, under certain conditions, also *in vitro*. The accelerating effect of methylene blue upon the rate of disappearance of methemoglobin *in vivo* has been confirmed by Wendel (244), and more recently by Bodansky and Gutmann (24). The latter also demonstrated that an intravenous dose of 1 mg. of methylene blue per kg. body weight is highly and rapidly effective in saving dogs from death at degrees of methemoglobinemia which would otherwise be fatal.

The use of ascorbic acid in the treatment of methemoglobinemia appears to have been introduced by Lian, Frumusan and Sassier (160) who, in observing two cases of congenital methemoglobinemia, reasoned that the concentration of methemoglobin represented a disturbance in the oxidation-reduction equilibrium of the blood pigment and that ascorbic acid should reduce the methemoglobin to hemoglobin. This surmise was confirmed in these two instances, and subsequent investigators have demonstrated this phenomenon and studied its quantitative aspects *in vitro*, both on crystallized methemoglobin and methemoglobinemic erythrocytes, and *in vivo* (24, 83, 155, 236). Bodansky and Gutmann (24) found that ascorbic acid was much less effective than methylene blue in accelerating the reduction of methemoglobin in dogs. At a dose of 200 mg. or 1.1 mM of ascorbic acid per kg., reduction of a maximal concentration of about 60 per cent to one-half value required 100 to 204 minutes. A much smaller dose of methylene blue, namely, 1 mg. or about 0.003 mM per kg. accomplished the same degree of reduction in the much shorter period of twenty-two to forty-two minutes.

Lian and his associates (160) injected intravenously 100 mg. of ascorbic acid daily for three weeks. The concentration of methemoglobin decreased from 45 to 26 per cent; the cyanosis diminished and the dyspnea and headaches disappeared. Discontinuation of therapy led to a rise in the methemoglobin concentration. Deeny, Murdock and Rogan (61) treated two cases with oral administration of ascorbic acid at doses which began at 50 mg. twice daily and were increased to maintenance levels of 150 and 200 mg. twice daily. The blood methemoglobin concentration in the first case decreased from an initial level of about 34 per cent to 11 per cent at the end of sixty-three days of treatment. In the second case, the methemoglobin concentration decreased from about 43 per cent to 6 per cent in one month and remained at about that concentration on a maintenance level of 200 mg. of ascorbic acid twice daily. There are several other reports of treatment of this condition by oral or parenteral administration of ascorbic acid (41, 85, 218).

Methylene blue has also been found to be effective in the treatment of familial idiopathic methemoglobinemia. King and his associates (150) found the oral administration of about 260 mg. daily to a patient weighing 52 kg. decreased the methemoglobin concentration from an initial concentration of about 35 per cent to less than 10 per cent in approximately a week; a daily oral dose of 65 mg. was necessary to maintain the methemoglobin concentration at less than 10 per cent. Eder, Finch and McKee (69) found in their patient that an intravenous injection of 90 mg. of methylene blue reduced the concentration of methemoglobin from an initial value of about 45 per cent to half this value in ten minutes and to negligible levels in thirty minutes. Physical activity was then possible without headaches or dyspnea. An oral daily dose of 240 mg. of methylene blue in enteric-coated tablets was well tolerated and kept the patient free of cyanosis for a period of several years. The polycythemia disappeared during the treatment.

Since familial idiopathic methemoglobinemia is apparently one of the group of diseases classifiable as "inborn errors of metabolism," the methemoglobinemia requires constant attention during life. Treatment is usually not an emergency since the patient will have had the disease for some time and since the disease is not particularly incapacitating. Intravenous therapy is, therefore, not necessary. Oral administration of ascorbic acid in daily, divided doses of 400 to 500 mg. or of methylene blue in similar doses of about 250 mg. should be adequate in reducing the methemoglobin concentrations to levels of about 5 per cent within a few weeks and in maintaining the methemoglobin at this low level so long as the administration is continued. One may envision the necessity for more prompt intravenous medication with methylene blue in the event that a patient with familial, idiopathic methemoglobinemia succumbs to pulmonary or cardiac disease, but the probability of this coincidence is, of course, extremely low.

D. Enterogenous methemoglobinemia

The literature contains a number of clinically ill-defined cases of methemoglobinemia which have been grouped together and designated as enterogenous cyanosis. These patients usually have abdominal pain accompanied either by diarrhea or constipation (76). The degree of methemoglobinemia may fluctuate widely; pronounced degrees of methemoglobinemia are associated with cyanosis, headache, dyspnea and, if very pronounced, dizziness and collapse. The methemoglobinemia has been variously considered as being due to the excessive presence of nitrite-producing bacteria in the gastrointestinal tract, to the ingestion of large amounts of aniline derivatives for relief of headache, or to a metabolic derangement resulting in the formation of a methemoglobin-forming p-quinone (76–78). If the methemoglobinemia is of moderate degree, the treatment is that described in the previous section.

E. Severe and Lethal Methemoglobinemias

When the degree of methemoglobinemia is so pronounced that semi-stupor or unconsciousness is present, an emergency exists and treatment must be prompt. As we have already indicated, such dangerous degrees of methemoglobinemia may result from industrial contact with potent methemoglobin-forming agents or from ingestion of compounds, solutions or food products containing such agents.

The chief feature of treatment consists in the intravenous injection of methylene blue in a dose of 1 to 2 mg. per kg. body weight. This dose is far below the toxic level. Since methylene blue may be readily made up in a 1 per cent aqueous or saline solution, the necessary amount may be introduced from a 10 cc. syringe in less than one minute. It is, therefore, not necessary, and indeed may delay treatment, to employ infusions of methylene blue in more dilute solution over a longer period of time. In cases of severe methemoglobinemia such general supportive measures as inhalation of oxygen or intravenous infusions of glucose, of glucose-saline, or of whole blood are often considered and sometimes used. But the data which we reviewed in an earlier section show that there is no physiological justification for their use, and attempts to institute them may only delay effective treatment. On the other hand, as we have already noted, the intravenous injection of methylene blue in a dose of 1 mg. per kg. is highly and rapidly effective in saving dogs from death at degrees of methemoglobinemia which would otherwise be fatal. There is ample clinical evidence to show that a similar situation holds in man.

William and Challis (243) reported a case of methemoglobinemia induced by contact with p-bromanilin and p-bromorthosulfanilic acid. The patient was intensely cyanotic and semi-comatose when 100 cc. of a 1 per cent solution, equivalent to about 15 mg. of methylene blue per kg., were injected. Within one hour, the cyanosis had disappeared and the patient had revived. The dose used was unnecessarily high, as subsequent clinical reports indicate, but this case incidentally illustrates the relatively non-toxic nature of methylene blue. Steele and Spink (225) reported two patients with marked methemoglobinemia;

one was disoriented and the other was semi-stuporous. The intravenous injection of 50 cc. of a 0.5 per cent solution of methylene blue (about 3 to 4 mg. per kg.) resulted in dramatic improvement. Within fifteen minutes after the injection, the patients sat up and appeared well. No quantitative methemoglobin determinations were done in these cases.

That even smaller doses of methylene blue are highly effective is demonstrated by recent reports on the treatment of nitrite-induced methemoglobinemia, due to the inclusion in the milk formula of well water which contained nitrate. Faucett and Miller (74) reported two instances of poisoning in infants, with marked cyanosis, increased respiratory and heart rates, and marked lethargy. One half cc. of a 1 per cent solution of methylene blue was injected intravenously into one infant who had a 58 per cent concentration of methemoglobin and 0.6 cc. into the second infant who had a methemoglobin concentration of 71 per cent. These amounts correspond to doses of about 1 to 2 mg. per kg. Within thirty minutes after the injections, the infants appeared clinically well; the respiratory and heart rates had decreased to normal and the methemoglobin concentrations were 4 to 5 per cent of the total blood pigment.

It is occasionally considered paradoxical that methylene blue which is a methemoglobin former should be so effective in decreasing methemoglobinemia (70). However, the evidence we have presented earlier in this review shows that this situation is really not paradoxical (22). For although methylene blue is indeed a methemoglobin former, it is not, contrary to the usual impression, a good methemoglobin former *in vivo* in man (182). In contrast, doses much smaller than those which produce methemoglobin are capable of reducing methemoglobin. In other words, when methylene blue is injected into man, two reactions ensue: 1) a direct oxidation of hemoglobin to methemoglobin, and 2) an opposing reaction, the reduction of methemoglobin to hemoglobin, for which coenzyme and coenzyme factor are necessary. Apparently, the latter reaction is much more effective, so that the equlibrium state between the two reactions is pitched at a point of very low methemoglobin formation. This equilibrium is arrived at whether methylene blue is injected in a person with no methemoglobinemia or in one with a high concentration of methemoglobin.

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PHARMACOLOGY OF THE CORONARY CIRCULATION

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I. INTRODUCTION

The coronary blood flow is the blood which enters the coronary ostia, irrigates the myocardium and drains into the right auricle and ventricle and to a small extent into the left ventricle. The determinants of the amount of coronary blood flow are the head of pressure, *i.e.*, the aortic pressure at the coronary ostia, and the resistance of the coronary bed. If the viscosity of the blood is constant, there are two components to the resistance of the coronary bed: (1) the intrinsic component which is the state of constriction or dilatation of the coronary vessels and (2) the extrinsic component which is the magnitude of the "extravascular support", *i.e.*, the effect that the myocardium exerts on the coronary bed during each systole by compressing the coronary bed from the outside and possibly also during diastole by the effect of its tonus. The part played in determining the resistance of the coronary bed by the pressure existing at the opening of the coronary vessels in the cardiac chambers into which they drain remains to be determined. A discussion of the physiological influences that control the aortic pressure and the magnitude of the extravascular support does not belong in this review and such influences will be mentioned when necessary during the presentation of the effect of drugs on the coronary circulation. The degree of constriction or dilatation of the coronary bed seems to be under nervous control but the metabolites locally produced probably also play a very important rôle in modifying the caliber of the coronary vessels. Probably all and certainly most of the drugs that modify the coronary circulation also affect the dynamics of the general circulation through the action they have on the myocardium and/or on the peripheral vessels. Therefore the knowledge of the effect of a drug on the coronary circulation must of necessity include not only the influence of such a drug on the volume of coronary blood flow and its mechanism or mechanisms of action, but also the effect of that drug on the work and metabolism of the heart.* Indeed what really matters is not the absolute amount of coronary blood flow, but the ratio between cardiac work and coronary blood flow; it is probable that certain drugs which, as will be seen later, increase the coronary flow dramatically, increase the work of the heart relatively more than the coronary flow. It is not known whether there ever are conditions or drugs that increase the work of the heart to such an extent that, even when they increase the coronary flow *pari passu*, the metabolic processes attending myocardial contraction cannot keep up with the increase in the cardiac work. Such a drug, of course, although increasing the coronary flow and keeping the ratio $\frac{\text{cardiac work}}{\text{coronary flow}}$ constant, would be detrimental to the heart. Finally, it should be added that the knowledge of the effect of a drug on the coronary circulation, to be complete, must include, besides its effect on the volume of coronary flow, on the ratio $\frac{\text{cardiac work}}{\text{coronary flow}}$ and on the metabolism of the heart, its effect on the efficiency of the heart. Indeed if a drug increases the efficiency of the heart, *i.e.*, increases the ratio $\frac{\text{mechanical work of the heart}}{\text{total energy liberated by the heart}}$, the heart when under the effect of such a drug will be able to perform more work with the same supply of blood and whatever the blood brings to the myocardium. The effect of such a drug will be tantamount to an increase in the amount of coronary blood flow. In short, a complete knowledge of the effect of a drug on the coronary circulation must include (a) the effect of that drug on the amount of coronary blood flow, (b) its mechanism or mechanisms of action, (c) its effect on the work of the heart and on the ratio $\frac{\text{cardiac work}}{\text{coronary flow}}$ as well as its effect on the metabolism of the heart, and (d) its effect on the efficiency of the heart. It will soon become obvious that our knowledge is far from complete.

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* Metabolism of the heart is used to embrace the nature and amount of the substances used by the mycardium as a source of energy and the nature and amount of the metabolites produced.

II. METHODS

Although the ultimate goal of the physiologist and pharmacologist is to know the effect of an agent or drug on the coronary circulation of the intact unanesthetized animal and eventually of man, it is necessary to resort to more simple preparations than the whole animal in order to analyze the mechanisms of action of physiological or pharmacological agents. It seems, therefore, appropriate at this time to review critically the main preparations and methods that have been used to study the physiology and pharmacology of the coronary circulation.

Meyer (130) is the first one to have studied the reaction to drugs of rings of coronary arteries bathed in oxygenated carbonate or borate Ringer, Locke or Tyrode solutions and defibrinated blood. Even if it is assumed that such rings of coronary arteries separated from their nervous connections and under very artificial conditions react to drugs the same way as they would in the whole animal, such experiments give no information about the effect of the drug on the smaller coronary vessels which are the main component of the coronary bed. Of course such experiments also give no information on the effect of the drug on the other determinants of the coronary flow, *i.e.*, the head of pressure and the extravascular support. In short, it is obvious that little or no information can be obtained from such a preparation as to the effect of a drug in the whole animal and the information obtained with this method is to be interpreted with great caution and concerns only a factor of small importance in the regulation of the coronary flow.

The perfusion of the coronary bed of a heart in ventricular stand-still or ventricular fibrillation is another technic that has been widely used. The consensus is that the best method of perfusing the coronary bed is that in which the coronary arteries are cannulated, although other methods described and analyzed below have been used (117, 139). Wiggers (185) has brought hearts of cats, dogs and rabbits to a standstill by perfusing the coronary bed with non-oxygenated sodium chloride solution, the cannulas being inserted into the mouth of one or both coronary arteries, and he has measured the coronary flow by measuring the overflow from the right auricle and ventricle. Hammouda and Kinosita (92) have used essentially the same technic in the arrested rabbit heart, as well as in the normally beating heart, the artificially driven heart and the heart in ventricular fibrillation. More recently, Katz, Jochim and Weinstein (105) have perfused under constant pressure the cannulated coronary arteries of the isolated dog heart in ventricular fibrillation with defibrinated and heparinized oxygenated dog blood and estimated the coronary blood flow by measuring the outflow of blood from the coronary sinus, the pulmonary artery and the aorta. The purpose of using such preparations as the arrested or fibrillating ventricles is of course to study the effect of a drug on the coronary bed itself without the interference of possible changes in the other determinants of the coronary flow brought about by the drug, such as changes in the extravascular support. It must be kept in mind, however, that in such preparations the reactivity of the nerves controlling the coronary circulation may deteriorate unless the cerebral circulation is maintained. Also it does not necessarily follow that, because the ventricles are at a standstill or fibrillating, the extravascular support of the coronary bed is constant, although it is said to have remained constant or nearly so in a series of experiments by Katz and Lindner (106) on the effect of an excess of sodium, potassium and calcium ions on the coronary circulation. In short, it seems logical to conclude that the data obtained by perfusing the coronary bed of hearts in ventricular standstill or fibrillation may give information about the possible mechanisms of action of a drug but give no information about its effect on the coronary circulation of the whole animal.

The third type of preparation used is the perfusion of the coronary bed in the isolated beating heart via a cannula inserted into the aorta. This technic was originated by Porter (139) and Langendorff (117). Porter (139), using this technic, has perfused the beating heart of cats with defibrinated blood under constant pressure and measured the outflow of blood from the venae cavae. Although the head of pressure is assumed to be constant in such a preparation, there is little doubt that the heart beat influences somewhat the perfusion pressure and that, if the drug under study affects the strength of the heart beat, the head of pressure may not remain constant. Furthermore the magnitude of the extravascular support may also change if the drug under study modifies the strength of the ventricular contraction. The second pitfall of the technic is that the aortic valves are not competent in every preparation and some of the perfusion fluid leaks back into the left ventricle (185), which may result in changes of the left intraventricular pressure and changes in the magnitude of the extravascular support. It need hardly be mentioned that in such a preparation that portion of the coronary flow which drains into the left ventricle cannot be measured. Criticizing this technic, Wiggers (185) also points out a third pitfall, namely, that the perfusion fluid may accumulate at times in the cavities of the right heart and not escape completely to the outside; thus a falsely low estimate of the coronary flow is obtained. Against the first pitfall of the method, Wiggers (185) recommends the use of the arrested heart and not the beating heart, but as pointed out above this is not completely satisfactory. To obviate the errors due to the leakage of fluid back into the left ventricle and the accumulation of fluid in the right cavities of the heart and in the left ventricle, Wiggers (185) suggests to drain the left ventricle or to cannulate the coronary arteries instead of perfusing them via an aortic cannula, and to drain widely the right cardiac chambers. As mentioned above, Hammouda and Kinosita (92) have also used the perfusion of the beating rabbit heart with oxygenated carbonate-buffered Ringer fluid and oxygenated borate-buffered Ringer fluid.

In the preparations mentioned above, the amount of coronary flow has been estimated by collecting and measuring the outflow from the right cardiac chambers, attention being paid to draining the right chambers freely. Brodie and Cullis (21) have used an ingenious plethysmographic method to record continuously the coronary outflow. Sassa (146) has used Atzler and Frank's method (14) to measure the coronary inflow; the method consists essentially in counting the number of air bubbles entering the constant pressure bottle which contains the perfusion fluid. Whatever the method of recording and/or measuring the amount of coronary flow may be, it seems that the perfusion of the coronary bed in the isolated beating heart as a method of approach to the study of the coronary circulation has very marked limitations for most purposes. Its greatest usefulness probably lies in the study of the intimate mechanism by which changes in heart rate and strength of the ventricular contraction may affect the coronary flow. The perfusion of the isolated heart in ventricular standstill or fibrillation probably abrogates to a great extent the interference of changes in the extravascular support and gives information about the effect of the agent under study on the coronary bed itself but it fails to give much information about the effect of an agent or drug on the coronary circulation of the whole animal.

A great step forward was made when the heart-lung preparation became reliable (101, 113). It has proven very useful in the study of the different factors that affect the coronary circulation because in the heart-lung preparation the heart beats in a fairly natural manner and one determinant of the coronary flow can be changed while all the others can be kept constant. A great deal of information about the physiology and pharmacology of the coronary circulation has been gathered with the heart-lung preparation which is more physiological than the preparations previously described and gives more and better information. However, it must be kept in mind that such a preparation is still very different physiologically from the whole animal. Therefore, the information gathered with this technic must be critically appraised when an attempt is made to integrate it into the physiology and pharmacology of the coronary circulation of the whole animal. For example, in the heart-lung preparation, although stimulation of the vagi and sympathetic nerves is still effective, the reflex nervous control of the coronary circulation is abolished since there is no cerebral circulation in the ordinary heart-lung preparation. It must also be remembered that those features of the heart-lung preparation that constitute its advantages are also its drawbacks. It is, of course, of great interest in the study of the effect of a physiological or pharmacological agent to be able, as in the heart-lung preparation, to keep blood pressure, heart rate and cardiac output and work constant and to conclude that the increase or decrease in coronary flow observed is due to a decrease or an increase, respectively, in the resistance of the coronary bed. Although observations made with the heart-lung preparation tell what an agent can do to the coronary flow and the coronary bed, they do not reveal what it actually does to the coronary flow or the coronary bed in the whole animal. It may well be that an agent or drug, when acting in the whole animal, has a direct effect on the coronary bed and flow which is neutralized or even offset by the indirect effect of the drug's action on the blood pressure, heart rate, cardiac output and cardiac work. In other words, to fulfill the four desiderata mentioned at the end of the introduction, experimentation on the whole animal is necessary to establish the effect of the drug on the coronary circulation, and the work, the metabolism and the efficiency of the heart, but experimentation on simpler preparations, such as the isolated heart and the heart-lung preparation, is necessary to establish the intimate mechanisms of action of the drug. Of these simpler preparations, the heart-lung seems to be the best and the most widely useful. As has been mentioned above, one objection to the heart-lung preparation is that in such a preparation there is no cerebral circulation which makes it impossible to study the central and reflex regulation of the coronary circulation. To obviate this objection, Anrep and Segall (12, 13) have designed the innervated heart-lung preparation which is an ordinary heart-lung preparation in which the heart and brain are supplied with blood from separate sources that can be controlled independently. The coronary blood supply comes from the aorta of the heart-lung preparation or from a perfusion flask whereas the cerebral blood supply comes from another heart-lung preparation, a pump or the circulation of another animal. In this manner the heart retains its connection with a living central nervous system. This preparation has all the advantages of the ordinary or denervated heart-lung preparation and also affords the opportunity to study the effect of central and reflex nervous factors on the coronary circulation.

The whole anesthetized animal has been used by numerous investigators (1, 2, 78, 79, 140, 171, 184). There is no doubt that the study of a physiological or pharmacological agent in the whole anesthetized animal is more likely than the previously described technics to reveal the effect of that agent in the unanesthetized animal or man, but it often reveals little as to the mechanism of action of the agent studied. Because of technical difficulties, in most experiments on the whole anesthetized animal the only available information, besides the amount of coronary flow, is the arterial blood pressure and the heart rate. From coronary flow, arterial blood pressure and heart rate it is difficult to infer much about the mechanism of action of the agent studied. During the period of action of a drug, for example, it is only when coronary flow and blood pressure vary in opposite direction or when either varies alone that something is learned about the mechanism of action of that drug. If a drug increases both coronary flow and arterial blood pressure, it is impossible to know whether the increase in flow is due to the increase in the head of pressure alone or whether the drug may also have decreased directly or indirectly the resistance of the coronary bed. When a drug lowers both flow and pressure, it is impossible to know whether the decrease in flow is accounted for by the decrease in the head of pressure alone or whether the drug has also increased the resistance of the coronary bed. However, if a drug decreases the blood pressure and the flow remains the same or rises, then the drug has decreased the resistance of the coronary bed. If a drug lowers the flow whereas the blood pressure remains the same or rises, there is no doubt that the drug has increased the resistance of the coronary bed. It must be added that, although in such favorable examples as just mentioned above, a conclusion can be reached as to what the effect of a drug is on the coronary flow and on the resistance of the coronary bed, this insight into the mechanism of action of the drug remains frustrating. Indeed, if under favorable circumstances the conclusion has been reached that a drug has increased the coronary flow by decreasing the resistance of the coronary bed, it still is impossible to know whether that decrease in the resistance is due to a decrease in the magnitude of the extravascular support or to a dilatation of the coronary bed. *A fortiori* is it impossible to determine whether the dilatation of the coronary bed, if dilatation there has been, is due to the effect of the drug directly on the blood vessel wall itself or indirectly on the blood vessels via nervous influences, either local or reflex in origin. It is even impossible to know whether such a dilatation may not be due to a relative ischemia of the myocardium (73), the drug increasing the cardiac work more than the coronary flow and the metabolites which accumulate locally being ultimately responsible for at least part of the dilatation of the coronary bed.

To measure the coronary flow in the preparations that have just been reviewed, numerous methods have been used. Some of the simpler and older ones have already been described. Great progress was made in the study of the coronary circulation when Morawitz and Zahn (132) designed a special cannula which, when inserted into the coronary sinus via the right atrium, drains the coronary sinus outflow. Numerous studies (5, 49, 125) seem to indicate that the volume of coronary sinus outflow amounts to about 60 per cent of the total coronary flow and bears a constant relationship to the total coronary flow under wide variations of cardiac output, aortic blood pressure and heart rate. If such is the case, the measure of the coronary sinus outflow is a very good approach to the study of the coronary circulation, the only objection being that the pressure at the end of the Morawitz-Zahn cannula is not the same as the pressure at the ostium of the coronary sinus; this may affect the amount of coronary sinus outflow. Although Anrep, Blalock and Hammouda (5) report that in most of their measurements the coronary sinus outflow represents between 60 and 67 per cent of the total coronary flow, they have found that in a few measurements the sinus outflow may vary from 48 to 71 per cent of the total coronary flow. Other investigators (104, 105, 109, 131, 166) have also been led to conclude that the ratio between coronary sinus outflow and total coronary flow is highly variable and therefore the sinus outflow is not a reliable index of the total coronary flow. Recently Gregg and Shipley (87), using better methods of measuring coronary inflow and coronary sinus outflow in the whole animal, have been able to show that under a wide variety of experimental conditions in which reasonably normal hemodynamic conditions prevail the relationship between coronary flow in the left coronary artery and coronary sinus outflow remains constant in the same animal. They have concluded that in any one experiment, when reasonably normal hemodynamic conditions prevail, changes in coronary sinus outflow can probably serve as a directional indicator of left coronary inflow and presumably also of total coronary inflow, although the actual volume of total coronary flow cannot be accurately predicted from the coronary sinus outflow since the relationship of coronary sinus flow to left coronary artery flow varies in different experiments. This very important question has obviously not been definitely settled and it would be most useful to have it settled before further work is done with technics based on the assumption that the sinus outflow bears a constant relationship to the total coronary flow. In 1925, Anrep and Downing (7) designed their anemometer to record the rate of coronary inflow when a cannulated coronary artery is perfused under constant pressure from a flask containing blood. The volume of blood flowing out of the flask causes the displacement of an equal amount of air through the upper part of the flask which contains a hot platinum wire. The air displacement cools the wire and the changes brought about in the electrical resistance of the wire by the cooling are recorded by a sensitive galvanometer. This instrument has given a great deal of information but can only be used when the coronary arteries are perfused from a constant pressure bottle. Furthermore, it has a considerable time lag and cannot record accurately rapid flow changes. Stehle (157), using a Langendorff preparation, has measured the coronary inflow by photographing the movement of a globule of toluene, colored deep red with iodine, placed in the inflow tube. Anrep, Davis and Volhard (6) have photographed the movement of a droplet of mercury placed in the blood stream. Both technics have a considerable lag but are of value when no accurate picture of the phasic changes of the coronary flow is desired. Wiggers and Cotton (187) have devised a technic consisting in the perfusion of a coronary artery with oxygenated Locke solution from a small reservoir connected to a chamber filled with air. Prior to each determination of flow, the air pressure is raised well above systolic aortic pressure and thus the perfusion fluid is driven into the coronary bed under a slowly declining pressure for a few beats. The flow is measured by recording the pressure changes that occur as the Locke solution enters the coronary artery, the slope of the curve giving the rate of coronary inflow. Although this technic has enlarged our knowledge of the dynamics of the coronary circulation, it has now been discarded for several reasons: the effect of the perfusion with Locke solution, even oxygenated, on the heart muscle; the constant changes of the perfusion pressure; the impossibility of having a continuous record; the necessity of using a perfusion pressure different from the natural one, quantitatively as well as qualitatively. In 1934, Soskin, Priest and Schultz (155) designed the first bubble flowmeter which has been recently used in the study of the coronary circulation by Eckenhoff, Hafkenschiel and Landmesser (36). This flowmeter is used to measure the mean blood flow by timing the speed of an air bubble of proper size injected into the blood stream when it passes through a glass tube of known volume inserted in the vascular bed under study. The air bubble serves to mark off the forward movement of the blood. This technic can only measure mean blood flow, it overestimates the flow if the bubble is too large or too small (22) and it cannot measure blood flow continuously.

In 1928, Rein (141) constructed the first thermostromuhr, which was later modified by Baldes and Herrick (15). It consists of a small diathermy unit, supplying a small and constant amount of heat, and two thermocouples. When the different parts, mounted into a compact unit, are snugly applied to an unopened blood vessel, part of the heat supplied by the diathermy unit is carried away by the stream of blood and the difference between the temperature of the two thermocouples, a function of the rate of the blood flow, is recorded by a sensitive galvanometer. Although the thermostromuhr only measures the mean blood flow, its advantages are great. The blood flow is measured in an unopened vessel. If it is desired, blood flow studies can be conducted on the unanesthetized animal since, after the thermostromuhr unit has been placed around the artery under anesthesia, the animal can be allowed to recover and used for several weeks thereafter. A few years ago, Gregg and his associates (78, 79, 83, 149) pointed out several sources of error in the thermostromuhr technic. It seems fair to state, however, that although the absolute values for blood flow obtained with the thermostromuhr must undoubtedly be interpreted with caution, the instrument can give a great deal of information within a wide range of physiological conditions. This is attested by the fact that none of the results or conclusions obtained with the thermostromuhr by the Mayo group has been disproven.

Green, Gregg and Wiggers (72) and Gregg, Green and Wiggers (82) have taken advantage of the fact that the rate of flow in a coronary artery at any instant is a function of the difference between the pressure in the central end of that coronary artery and the pressure in its peripheral end. Since the central coronary pressure is essentially similar to the aortic pressure (186), these authors record the aortic pressure near the coronary ostia as the central coronary pressure. The peripheral coronary pressure is recorded from a side branch of one of the main coronary arteries. The curve obtained by subtracting the corrected peripheral pressure curve from the aortic pressure curve represents, at any instant, the instantaneous rate of flow and the area beneath the curve obtained during a cardiac cycle gives an index of the volume of coronary flow. The assumptions upon which this "differential pressure method" is based have not proven to be entirely valid and it seems that the method under certain circumstances may neither indicate the directional shifts of flow nor represent the magnitude of the changes in flow. While such a procedure has added to our knowledge, it must be discarded as a method of quantitating the coronary flow (79). In 1940, Gregg and Green (81) described a recording orifice meter. It is based on the fact that if the lateral pressure is recorded above and below a point of constant constriction in a flowing stream, the difference in the pressures which can be directly recorded with a differential manometer is a function of the rate of flow. This technic permits a continuous recording of the phasic changes of coronary flow. However, when the value of the mean coronary flow is desired, the differential pressure curve must be redrawn on a linear ordinate scale before it can be integrated, which involves a great deal of tedious work. Nevertheless this technic has added a very great deal to our knowledge of the coronary circulation.

The rotameter, first used in 1942 to measure blood flow (88), consists essentially in a vertical tube with tapered bore in which there is a small, freely movable float the height of which varies with the rate of flow. It has been modified by Shipley and Crittenden (26, 147) to permit continuous recording of the flow. It measures only mean blood flow but it is accurate, easy to use and is probably one of the best methods to record mean blood flow.

Recently the nitrous oxide method, first used to measure cerebral blood flow (110), has been used to measure the coronary blood flow through the left ventricle (35). It is based on the Fick principle, *i.e.*, the flow of blood through an organ per unit of time is equal to the amount of a substance taken up from the blood by that organ per unit of time divided by the difference between the concentration of that substance in the arterial blood and its concentration in the total venous blood drained from that organ during that time. In the nitrous oxide method as applied to the measurement of the coronary blood flow, the arterial nitrous oxide concentration is determined on blood drawn from any available artery and the venous nitrous oxide concentration is determined on blood drawn from the coronary sinus without contamination. The amount of coronary blood flow through those parts of the heart whose venous drainage occurs via the coronary sinus is equal to the nitrous oxide uptake of the heart divided by the arterio-venous nitrous oxide difference. The numerator of this fraction is assumed to be equal to the concentration of nitrous oxide in the venous blood after equilibrium between blood and heart tissue has been established multiplied by a partition coefficient for nitrous oxide between blood and heart tissue. The denominator is obtained by computing the integrated difference between the concentrations of nitrous oxide in arterial and venous blood during the period of equilibration, during which the animal inhales a mixture of nitrogen and oxygen containing 15 per cent of nitrous oxide. From the formula it is easy to obtain the amount of coronary blood flow per minute per 100 grams of that part of the myocardium whose venous drainage occurs via the coronary sinus, *i.e.*, per 100 grams of left ventricle. The method can be used in lightly anesthetized or even unanesthetized animals which are in conditions closer to normal than in any other method except the thermostromuhr method. The agreement between figures for coronary flow obtained by the nitrous oxide method and by the bubble flowmeter is good (35); but Green, Gregg and Czerwonka (75) have been unable to obtain agreement in experiments in which they have measured the coronary flow simultaneously with a rotameter and the nitrous oxide method. A disadvantage of the nitrous oxide method is that it can be used only if the conditions under which the flow is measured remain constant for at least the ten minutes required for the procedure. This precludes its use under circumstances in which rapid fluctuations of flow occur. The pitfalls of contamination of the coronary sinus blood by atrial blood, of occluding the venous drainage and of trauma to the coronary system cannot be disregarded. Finally, the effect of nitrous oxide itself on the coronary flow has not been determined.

Because relatively little information is obtained when only arterial blood pressure, heart rate and coronary flow are measured, several authors have recently tried to measure not only coronary flow, arterial blood pressure and heart rate but also cardiac output in the whole anesthetized animal. Eckenhoff, Hafkenschiel and Landmesser (36) have measured arterial blood pressure, heart rate, cardiac output and coronary flow, the latter two by the Fick method and the bubble flowmeter, respectively. Eckenhoff, Hafkenschiel, Landmesser and Harmel (37) have simultaneously determined coronary flow, arterial blood pressure, heart rate, cardiac output, cardiac work and cardiac efficiency. Very recently, Wégria, Frank, Misrahy, Sioussat, Sommer and McCormack (176–178) have designed a technic by which the output of the left ventricle, the coronary blood flow, the mean arterial blood pressure and the heart rate can be simultaneously and continuously recorded in the whole anesthetized animal, the flows being recorded by the simultaneous use of two rotameters, one for the coronary flow and one for the cardiac output. In addition the cardiac work can be calculated from the data obtained. Although such technics should prove valuable, it must be kept in mind that the anesthetized animal, especially with open chest, under artificial respiration and having undergone varied operative procedures, may well be different in its reactions from the unanesthetized animal and human being, quantitatively and possibly even qualitatively. Unpublished data (172) seem to indicate, for example, that vasodilator drugs lower the arterial blood pressure more markedly in the anesthetized than in the unanesthetized dog. It has also been our observation that the type of anesthetic used may make a difference in the type of reaction observed (172). For example, in a dog under chloralose anesthesia, a dose of nitroglycerin may lower the arterial blood pressure very markedly and still increase the coronary flow, whereas a smaller dose of nitroglycerin given to a dog under morphine-barbital anesthesia may lower the blood pressure less markedly and yet the coronary flow will be markedly decreased. These quantitative and sometimes qualitative differences between the effects of physiological and pharmacological agents on the coronary circulation, depending upon the type of anesthetic used, are not surprising since most anesthetics are known to influence markedly the cardiovascular system and its regulatory mechanisms. For example, sodium pentobarbital has been shown to have a marked effect on the coronary circulation (47). However, it is comforting that, in a study of the effect of a large number of drugs on the coronary circulation in a series of dogs anesthetized with chloralose (175) and in a series of unanesthetized dogs (47), no qualitative difference has been observed between the two groups. To obviate the objection of anesthesia and operative procedures, some work has been done on the unanesthetized animal with the thermostromuhr (47). The part of the thermostromuhr which has to be placed around the coronary artery, *i.e.*, the thermocouple unit, is applied under general anesthesia and the insulated wires connecting the thermocouple unit with the recording part of the thermostromuhr are brought through the skin. The dog is allowed to recover and can be used for a certain number of days or even weeks. Recently the nitrous oxide method has been used in unanesthetized human beings to study coronary circulation (16).

It is obvious from what has been said that it is difficult to study experimentally the effect of a physiological or pharmacological agent on the different functions that must be taken into consideration when a complete knowledge of the effect of that agent on the coronary circulation is to be gained. Even when this is achieved on the unanesthetized animal, there always remains the possibility that the reactions observed in the coronary circulation of the animal may be different from those in the coronary circulation in man. For these reasons, there have been many attempts to design methods to evaluate indirectly the effect of physiological or pharmacological agents on the human coronary circulation. The most widely used approach to the problem has been to study the effect of the agent, generally a drug, on anginal pain (143). Since anginal pain is probably due to anoxia of the myocardium caused by an absolute or relative ischemia of the myocardium, it is assumed that if a drug prevents or relieves angina it does so by preventing or relieving the ischemia. Although such an approach when judiciously used can give a great deal of practical information, it reveals little about the mode of action of the drug and from such data it is impossible to know whether the drug is effective by increasing the coronary flow, by decreasing the work of the heart, by increasing the efficiency of the heart or by a combined action. Neither can it be determined whether an increase of coronary flow, if such occurs, is only relative to an increase in the work of the heart or whether the drug acts only by increasing coronary flow without modifying the work of the heart (181). Another objection to this method is that it offers more technical difficulties than would appear. The validity of the results obtained depends upon the study of the patient during a long control and experimental period. It must be remembered that the nature of coronary sclerosis, the main cause of relative coronary insufficiency, is often such that, as collateral circulation takes over the rôle of the narrowed vessel, the frequency of the anginal attacks decreases spontaneously and the pain may even disappear. The same may be true after the offending vessel has become completely occluded. Also there is a stage during the evolution of the disease at which the severity of the coronary insufficiency is such that even drugs of proven value do not affect the frequency or the intensity of the attacks. On the other hand, there are patients whose coronary insufficiency is so slight that the psychic effect of taking a placebo seems sufficient to decrease the frequency of the attacks. In short, if the effect of a drug on the anginal attacks in patients with coronary sclerosis is to be tested, the method is not so easy as it first appears and even if it gives practical information about the usefulness of a drug, it reveals nothing about its mode of action. However, such clinical tests must ultimately be done on drugs which pharmacological studies indicate to be promising. In recent years, clinical tests have been used to reveal a latent coronary insufficiency. These tests consist essentially in exerting the patient by different types of exercise (127) or subjecting the patient to a certain degree of anoxic anoxia by making him breathe a mixture of nitrogen and oxygen containing approximately 10 per cent oxygen (138). In these tests, the appearance of anginal pain and/or characteristic electrocardiographic changes during the period of exertion or anoxia is an index of latent coronary insufficiency. Drugs have been studied for their ability to prevent or delay the appearance of pain and/or electrocardiographic changes in these tests (120, 144, 189). Although exercise and anoxemia tests have the advantages of avoiding long periods of observation and of giving information of direct clinical applicability, they reveal nothing about the mechanisms of action of the drug. Recently, Wégria, Segers, Keating and Ward (182) have determined the minimal restriction of coronary flow which induces electrocardiographic changes in the ischemic area of the myocardium. Whether such a preparation may lend itself to the study of drugs remains to be determined but it is conceivable that a drug might abolish the electrocardiographic changes due to the ischemia by modifying the metabolism of the ischemic area and/or by dilating the coronary bed distal to the constriction; this technic may prove to be a valuable approach.

To conclude this review of the methods used to study coronary circulation, it should again be stated that to obtain exact knowledge of the effect of a drug on the coronary circulation as defined above, it is necessary to use a gamut of methods of approach, extending from a study of the effect of a drug on the coronary circulation of simple preparations, such as the isolated heart in ventricular fibrillation or standstill, to a study of its effect on angina in human beings; each of the technics constitutes a necessary step toward the ultimate goal.

III. PHYSIOLOGY

It seems indispensable to consider briefly the physiology of the coronary circulation before discussing its pharmacology. In this section will be discussed the determinants of the coronary flow, their respective rôles and how they are modified by cardiac or extracardiac influences. It will thereafter be much easier to approach rationally the pharmacology of the coronary circulation.

A great deal of information is obtained from an inspection of tracings representing phasic changes of coronary flow during a cardiac cycle, as recorded by an adequate flowmeter such as the orifice meter (73, 81). Although such flow tracings vary under different hemodynamic conditions and although there are differences between the patterns of flow in the left and right coronary arteries, the coronary flow tracings present certain constant essential features. At the onset of the isometric contraction of the ventricles, there is an abrupt decrease of the coronary flow and during this phase there may even be a flow of blood backward from the coronary arteries into the aorta. This decrease of flow which occasionally culminates in a temporary backflow is undoubtedly due to the compression exerted on the coronary bed by the contracting myocardium. As the aortic valves open and the pressure rises in the aorta, the forward flow of blood is resumed if a backflow has occurred during the isometric contraction, or the coronary flow suddenly increases if the flow has only decreased during the isometric contraction. When the pressure in the aorta decreases after having reached a peak, the coronary flow decreases. The changes that occur in the aortic pressure during systole explain adequately the fluctuations of the coronary flow at this stage. From protodiastole on, the ventricular myocardium relaxes, the compression of the coronary bed by the myocardium decreases, the intensity of the extravascular support of the coronary bed reaches its minimum and, as a result, the coronary flow increases markedly and abruptly. Although the pressure in the aorta decreases steadily due to the run off of blood towards the periphery, the coronary flow remains high but slowly declines, however, as is to be expected from the decrease in the head of aortic pressure. At the onset of the isometric contraction of the ventricles, the sudden decrease of flow occurs as mentioned above. This flow pattern has been well established and all investigators agree that it is characteristic of the flow of blood at least in the large coronary arteries. From this pattern it is relatively simple to appraise, at least qualitatively, the influence of factors such as heart rate and aortic blood pressure on the coronary flow. To do this, as well as to determine the effect of a drug on the coronary flow, two types of experiments are necessary. The first type of experiment is performed on simple preparations such as the isolated heart, and conditions are set so that there is only one variable, *i.e.*, the variable whose influence on the coronary flow is being studied. This type of experiment can reveal what a change in heart rate does to the coronary flow, but it does not reveal what a change in heart rate does to the coronary flow in the whole animal. Therefore the effect of changes in heart rate on the coronary flow must also be studied on the whole animal or on equivalent preparations.

Heart rate. Under essentially normal cardiovascular conditions, more coronary flow occurs during diastole than during systole. Therefore it is to be expected that within limits an increase in heart rate *per se* will decrease the amount of coronary flow per minute and a decrease in heart rate will increase it, which is what Anrep and Häusler (9) have found on the isolated heart perfused under constant pressure. However, if the rate of the heart is such that each beat becomes very weak, the decrease in coronary flow due to the shortening of diastole may be offset by the decrease in the restriction of flow due to systole;

instead of decreasing as the heart rate increases, the coronary flow may then remain unchanged or even increase. In experiments by Anrep and King on the denervated heart-lung preparation (11) and by Anrep and Segall on the innervated heart-lung preparation (13), changes in heart rate within wide limits have been found to have no effect on the coronary flow, whereas Hausner, Essex, Herrick and Baldes (94) have described an increase in coronary flow with an increase in heart rate in the denervated heart-lung preparation. Recently Wégria and Keating (18) have reported that an increase in heart rate in the anesthetized dog produces a temporary decrease in the coronary flow, followed by a rise in the coronary flow above control level, within wide limits of tachycardia. If the rate of the tachycardia is very high, the coronary flow decreases and then only comes back to the control level, and sometimes remains below it. In short, most investigators are agreed that a rise in heart rate *per se* tends to reduce the coronary flow since it increases the period of lower coronary flow (systole) and decreases the period of higher coronary flow (diastole). However, there are some experimental data which do not substantiate this view. Most investigators hold that changes of heart rate *per se*, over a wide range of rates, do not influence the coronary flow; at least, they agree that, in the whole animal, the effect which a change in heart rate must have on the coronary flow is compensated for or offset by the other cardiovascular effects of the drug or the agent responsible for the change in heart rate.

Aortic blood pressure. Although it has been shown that a rise in the aortic pressure, by increasing the strength of the ventricular contraction, tends to decrease the coronary flow by increasing the restriction of flow during systole (2), *a priori* considerations about the pattern of the coronary flow throughout the whole cardiac cycle, as well as all the available experimental data, confirm that a rise in the aortic blood pressure *per se* results in an increase of coronary blood flow and that a decrease of aortic pressure leads to a decrease in the coronary flow (2, 94, 125). Smith, Miller and Graber (153) believe that the diastolic pressure is the most important but Anrep and King (11) point out that diastolic pressure must be understood as mean diastolic pressure if the view of Smith, Miller and Graber is to be accepted. The effect of an increase of aortic pressure on coronary flow via its effect on cardiac work is discussed below.

Cardiac output and work. Markwalder and Starling (125) were the first investigators to show that changes in the output of the heart in a denervated heart-lung preparation do not modify the coronary sinus outflow as long as the mean arterial blood pressure remains constant. The problem has been reinvestigated by Anrep and Segall (13) in the denervated and the innervated heart-lung preparation. They have concluded that in the denervated heart-lung preparation the arterial blood pressure is the only mechanical factor which determines the coronary flow and that neither a change in heart rate nor a change in systolic output nor a change in minute output have any influence *per se* on the coronary flow. In the innervated heart-lung preparation, on the contrary, the cardiac output is an important determinant of the coronary flow, an increase in output producing a rise in the coronary flow. This influence is thought to be of reflex instead of decreasing as the heart rate increases, the coronary flow may then remain unchanged or even increase. In experiments by Anrep and King on the denervated heart-lung preparation (11) and by Anrep and Segall on the innervated heart-lung preparation (13), changes in heart rate within wide limits have been found to have no effect on the coronary flow, whereas Hausner, Essex, Herrick and Baldes (94) have described an increase in coronary flow with an increase in heart rate in the denervated heart-lung preparation. Recently Wégria and Keating (18) have reported that an increase in heart rate in the anesthetized dog produces a temporary decrease in the coronary flow, followed by a rise in the coronary flow above control level, within wide limits of tachycardia. If the rate of the tachycardia is very high, the coronary flow decreases and then only comes back to the control level, and sometimes remains below it. In short, most investigators are agreed that a rise in heart rate *per se* tends to reduce the coronary flow since it increases the period of lower coronary flow (systole) and decreases the period of higher coronary flow (diastole). However, there are some experimental data which do not substantiate this view. Most investigators hold that changes of heart rate *per se*, over a wide range of rates, do not influence the coronary flow; at least, they agree that, in the whole animal, the effect which a change in heart rate must have on the coronary flow is compensated for or offset by the other cardiovascular effects of the drug or the agent responsible for the change in heart rate.

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Nervous influences. The coronary blood vessels are richly supplied with nerves (190). Degeneration experiments have showed that the larger arterial branches are innervated by vagal and sympathetic fibers but mostly by sympathetic nerves whereas the smaller branches receive fibers mostly of vagal origin. No nerve supply to the capillaries has been demonstrated. It is very difficult to assess the rôle of nervous influences on the coronary circulation because the usual technics used to study nervous influences, such as stimulation and section of the nerves under study, besides exerting their effect on the coronary bed itself, also have numerous cardiodynamic effects such as changes in heart rate, blood pressure and cardiac output. Even when this obstacle is overcome, the effect proven by stimulation or section of a nerve only reveals what such a nerve can do but not what the rôle of that nerve actually is. In attempting to study the effect of sympathetic and parasympathetic nerves on the coronary bed, it is also difficult to gain information by the use of the corresponding chemical mediators, epinephrine, sympathin or acetylcholine, for the same reasons.

From their experiments on the innervated heart-lung preparation, Anrep and Segall (13) have concluded that the vagi exert a tonic constrictive influence on the coronary vessels, since section of the vagi in the cervical region increases the coronary flow even when heart rate, cardiac output and aortic blood presorigin since it is abolished by section of the vagi (13). Recently Gregg, Pritchard, Shipley and Wearn (84) have showed that a rise in the right ventricular pressure produced by pulmonary constriction, which results in an increase in the work and the metabolism of the heart, increases the blood flow in the right and the left coronary arteries. Gregg and Shipley (36) have also observed that a rise in the left ventricular pressure caused by a constriction of the aorta central to the coronary ostia, which increases the work and the metabolism of the heart, increases the blood flow in the left coronary artery. The same effects are also observed after section of the vagi and of the cardiac nerves from the sympathetic chain. The increase in flow is accompanied by a more complete oxygen desaturation of the coronary venous blood. It is thought by these investigators that the increase in coronary flow due to the increase in cardiac work is the result of an increased local production of metabolites and/or the appearance of a local relative anoxia. These experiments have also confirmed the conclusions of Anrep and Häusler (8) that, although a rise in the aortic pressure tends to decrease the coronary flow by increasing the magnitude of the extravascular support during systole, this effect is offset by the other mechanisms producing an increase in the coronary flow, the net result being an increase in the coronary flow. The coronary flow is also increased when the increase in the work of the heart is due only to an increase in the cardiac output. This was first demonstrated by Anrep and Segall (13) on the innervated heart-lung preparation. In conclusion, it seems that all investigators are generally agreed that an increase in the work of the heart *per se* does not modify the coronary flow in the denervated heart-lung preparation if the mean arterial blood pressure remains unchanged. In the innervated heart-lung preparation and in the whole animal, an increase in the work of the heart increases the coronary flow.

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That the vagi play an important rôle in the regulation of the coronary circulation seems to be shown by the fact that an increase of the cardiac output *per se* increases the coronary flow in the innervated heart-lung preparation only if the vagi are intact (13). Similar results confirming the rôle of the vagi have been reported by Rein (142) using his thermostromuhr on the whole animal. In experiments on the innervated heart-lung preparation, Anrep and Segall (13) have showed that the sympathetic nerves contain fibers which, when stimulated electrically or reflexly, produce a dilatation of the coronary bed. It must be added, however, that the conclusions reached by Anrep and Segall (13) as to the influence of the vagi and sympathetic nerves on the coronary circulation are valid only if no change in the extravascular support occurred during their experiments. It is true that in the experiments of these authors the aortic blood pressure and the cardiac output did not change but the volume of the heart may have changed during nerve stimulation or after nerve section, thereby possibly changing the magnitude of the extravascular support, in which case the effects ascribed to coronary constriction or dilatation may well have been due to increase or decrease of the extravascular support. This however, appears improbable, and it seems most likely that the conclusions reached by Anrep and Segall (13) in their classic studies are correct. Gregg and Shipley (85) using the orifice-meter and the rotameter in the anesthetized dog have found that electrical stimulation of the stellate ganglia and/or the cardiac nerves therefrom increases the coronary flow without necessarily changing aortic blood pressure or heart rate, from which they conclude that the sympathetic nerves when stimulated produce coronary vasodilatation. However, it must be pointed out that changes in the extravascular support have not been ruled out. Moreover, since these authors did not measure the changes in cardiac output that may have occurred during sympathetic stimulation, it may well be that the probable vasodilatation observed during stimulation of the sympathetic nerves is due to an increase in the output and the work of the heart. In a second paper on the same subject, Shipley and Gregg (148) have indeed found that in such experiments cardiac output, work and metabolism are increased. They have finally concluded that simulation of the sympathetic nerves to the heart produces dilatation of the coronary bed but have been unable to prove that such stimulation produces coronary dilatation directly, because it also increases cardiac output, work and metabolism; these changes afford an adequate explanation for the coronary dilatation. The experiments of Anrep and Segall (13), however, seem to indicate clearly, with the qualification mentioned above about changes in the extravascular support, that the vagi have a tonic vasoconstrictive influence on the coronary bed and that the sympathetic sure are kept constant. These vasomotor coronary fibers are much less sensitive to atropine than are the cardioinhibitory fibers. Stimulation of the peripheral end of the vagi furthermore decreases the coronary flow and this seems to be due to coronary constriction (13). The same authors have also proved that stimulation of the carotid sinus by a rise in the cephalic pressure lowers the coronary flow by producing constriction of the coronary bed; this has been confirmed by Stella (158). That the vagi play an important rôle in the regulation of the coronary circulation seems to be shown by the fact that an increase of the cardiac output *per se* increases the coronary flow in the innervated heart-lung preparation only if the vagi are intact (13). Similar results confirming the rôle of the vagi have been reported by Rein (142) using his thermostromuhr on the whole animal. In experiments on the innervated heart-lung preparation, Anrep and Segall (13) have showed that the sympathetic nerves contain fibers which, when stimulated electrically or reflexly, produce a dilatation of the coronary bed. It must be added, however, that the conclusions reached by Anrep and Segall (13) as to the influence of the vagi and sympathetic nerves on the coronary circulation are valid only if no change in the extravascular support occurred during their experiments. It is true that in the experiments of these authors the aortic blood pressure and the cardiac output did not change but the volume of the heart may have changed during nerve stimulation or after nerve section, thereby possibly changing the magnitude of the extravascular support, in which case the effects ascribed to coronary constriction or dilatation may well have been due to increase or decrease of the extravascular support. This however, appears improbable, and it seems most likely that the conclusions reached by Anrep and Segall (13) in their classic studies are correct. Gregg and Shipley (85) using the orifice-meter and the rotameter in the anesthetized dog have found that electrical stimulation of the stellate ganglia and/or the cardiac nerves therefrom increases the coronary flow without necessarily changing aortic blood pressure or heart rate, from which they conclude that the sympathetic nerves when stimulated produce coronary vasodilatation. However, it must be pointed out that changes in the extravascular support have not been ruled out. Moreover, since these authors did not measure the changes in cardiac output that may have occurred during sympathetic stimulation, it may well be that the probable vasodilatation observed during stimulation of the sympathetic nerves is due to an increase in the output and the work of the heart. In a second paper on the same subject, Shipley and Gregg (148) have indeed found that in such experiments cardiac output, work and metabolism are increased. They have finally concluded that simulation of the sympathetic nerves to the heart produces dilatation of the coronary bed but have been unable to prove that such stimulation produces coronary dilatation directly, because it also increases cardiac output, work and metabolism; these changes afford an adequate explanation for the coronary dilatation. The experiments of Anrep and Segall (13), however, seem to indicate clearly, with the qualification mentioned above about changes in the extravascular support, that the vagi have a tonic vasoconstrictive influence on the coronary bed and that the sympathetic nerves contain vasodilating fibers to the coronary bed. It must be added that Katz and Jochim (103), using the perfused isolated heart in ventricular fibrillation with normal cerebral circulation, have found that in the dog the vagi carry only tonically active cholinergic coronary vasodilator fibers; the cardiac sympathetic nerves carry adrenergic coronary dilator and adrenergic coronary constrictor fibers, both of which are tonically active, the tonic action being predominantly vasoconstrictor.

It is probable that part of the regulation of the coronary circulation is reflex in origin. The effect of an increase in the cerebral pressure (13) and in the pressure within the carotid sinus (158) has already been mentioned. For a number of years, it has been postulated by clinicians, probably from analogy with the peripheral circulation, that ligation or occlusion of one coronary branch can produce reflex constriction in other coronary branches (118, 124, 128). Although such reflex constriction has never been adequately proven (36, 79, 136), its existence has not been excluded. The effect of electrical stimulation of various afferent nerves upon the coronary circulation has been studied by several investigators but the significance of such studies remains to be determined. Greene (76), for example, has followed the changes in coronary flow produced by stimulation of the central end of cut sciatic, splanchnic and phrenic nerves and found an increase in coronary flow with weak stimuli but frequently a decrease with stronger stimuli. Electrical stimulation of afferent nerves may result in a diphasic effect on the coronary flow, first an increase then a decrease. Whether such changes are due to coronary dilatation and constriction remains to be proven. Distention of the stomach, the gallbladder and the esophagus has been claimed to increase the coronary flow while the arterial blood pressure rises above, remains at or falls below control level (100). On the other hand, Eckenhoff, Hafkenschiel and Landmesser (36) in a recent investigation of the effect of distention of the gallbaldder on coronary flow have found that the changes are variable and always in the same direction as the changes in blood pressure, the most common response being a decrease in coronary flow and blood pressure with an increase in heart rate. Gilbert, LeRoy and Fenn (58) have observed that distention of the stomach or peritoneal cavity in the anesthetized dog often causes a decrease in coronary flow while the blood pressure rises or remains unchanged. It has also been shown (57) that in the decerebrate dog stimulation of the nasal mucous membrane with ice water decreases coronary flow. Pulmonary embolus has been postulated to produce a reflex coronary vasoconstriction (29) but this has not been proven.

Asphyxia, anoxia, hypercapnia and myocardial ischemia. Certain chemical changes in the blood have been shown to affect the coronary circulation very markedly. Asphyxia (73) produced by shutting off the artificial respiration in the open chest dog increases the coronary flow within a few seconds in the absence of any changes in blood pressure or heart rate. Therefore this increase in coronary flow must be due to a decrease in the resistance of the coronary bed, brought about by dilatation of the coronary bed and possibly also by a decrease in the magnitude of the extravascular support (73). Of course if asphyxia lasts nerves contain vasodilating fibers to the coronary bed. It must be added that Katz and Jochim (103), using the perfused isolated heart in ventricular fibrillation with normal cerebral circulation, have found that in the dog the vagi carry only tonically active cholinergic coronary vasodilator fibers; the cardiac sympathetic nerves carry adrenergic coronary dilator and adrenergic coronary constrictor fibers, both of which are tonically active, the tonic action being predominantly vasoconstrictor.

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Complete ischemia of a portion of the myocardium produced by temporarily preventing blood from entering an area irrigated via a coronary branch, such as the ramus descendens anterior, induces a marked increase in the coronary flow to that area (36, 73, 107), even if the ischemia lasts only a few seconds. This increase in flow is due exclusively to a decrease in the resistance of the coronary bed. The same effect has been found to occur when the ischemia is only partial (182).

In summary, it seems that the effect of hypercapnia on the coronary circulation is disputed by different groups of investigators. In the experiments in which an excess of CO_2 increases the coronary flow, the increase is slight compared to that caused by anoxia. On the other hand, asphyxia, systemic anoxia produced by the inhalation of gas mixtures low in oxygen, local anoxia due to the intracoronary administration of cyanide and partial or total ischemia of the myocardium all increase the coronary flow markedly before or without inducing any change in blood pressure or heart rate. This increase in flow must, therefore, be due to a decrease in the resistance of the coronary bed. It seems probable that the decrease in the resistance of the coronary bed is due to dilatation of the vascular bed but it remains possible that a decrease in the extravascular support contributes to the decrease in the coronary resistance (73). The intimate mechanisms by which the resistance of the coronary bed is decreased are far from certain but several possibilities have been suggested: anoxia in the whole animal and in the innervated heart-lung preparation increases cardiac work (13, 84, 86) which through reflex influences and/or chemical influences locally in the myocardium, as mentioned above, leads to increased coronary flow; myocardial anoxia and ischemia cause the local production and accumulation of vasodilator metabolites such as adenosine and adenylic acid (169), local changes in pH (36) and local production of histamine (4).

Coronary venous pressure. A priori, it might be anticipated that, when the pressure increases on the venous side of the coronary bed, the flow of blood in the coronary arteries would decrease if no compensatory mechanism sets in. Experiments on the isolated dog heart (164) have indeed established that a decrease in the pressure gradient between coronary arteries and veins reduces the coronary flow. It is a difficult problem to study in the whole animal because, if the pressure on the venous side of the coronary bed is raised by increasing the pressure in the right chambers of the heart either by increasing the venous return of blood or by constricting the pulmonary artery, important cardiodynamic changes and compensatory mechanisms are evoked. These compensatory mechanisms may mask the intrinsic effect of the increase in the coronary venous pressure and make difficult the interpretation of the results observed, as will be remembered from what was said above about the effect of an increase in cardiac output and work on the coronary circulation. Furthermore, unless all the coronary artery blood flow and all the coronary venous outflow are measured in experiments on the whole animal, the conclusions drawn from the results obtained are hazardous because of the possibility of shunts opening between the different coronary arteries and between the different venous channels. For these reasons little is known about the effect of changes, especially moderate changes, in the coronary venous pressure on the coronary circulation in the whole animal. Acute occlusion of the coronary sinus, although causing a considerable rise of pressure in the coronary sinus (80), reduces the blood flow in the left coronary artery only very slightly, an average of 8 per cent (87). When the anterior cardiac veins are acutely occluded, the right coronary flow decreases 0 to 63 per cent, an average of 21 per cent. These experiments must not necessarily be interpreted as meaning that an increase in the pressure on the venous side of the coronary bed may have little effect on the coronary arterial inflow. They more probably mean that there exist many collateral communications between the different venous draining channels. In short, the effect of changes in the coronary venous pressure on the coronary artery inflow remains to be defined.

Digestion and exercise. Essex, Herrick, Baldes and Mann (41) have shown that digestion increases the coronary flow in the unanesthetized dog. The same authors have also studied the effect of exercise on coronary blood flow, mean arterial blood pressure and heart rate in the unanesthetized dog (43, 44) and observed that exercise increases coronary flow, blood pressure and heart rate but that the change in coronary blood flow follows that in heart rate much more closely than that in blood pressure. Bilateral cardiac sympathetic ganglionectomy from the eighth intercostal space anteriorly and including the stellate ganglion does not significantly alter the reactions of coronary flow, blood pressure and heart rate to exercise. After bilateral cervical vagotomy, exercise increases the coronary flow in those animals in which the exercise raises the blood pressure; in those dogs in which exercise does not increase the blood pressure, the coronary flow remains essentially unchanged. After bilateral vagotomy, exercise produces only a slight acceleration of the heart.

Temperature. Mart and Miller (126) raised the temperature of the right ventricle about 6°F. by applying diathermy over the heart and could not show any significant change in coronary flow. On the other hand, Cruickshank and Subba Rau (27) have found that isolated coronary arteries of ox, dog and man relax on cooling and contract on warming when the temperature varies between 27 and 38°C., a finding which has been confirmed by Kountz (114). As pointed out before, however, the results of such experiments are difficult to evaluate in terms of coronary circulation. Anrep and Häusler (9) have reinvestigated this problem on the heart-lung preparation by perfusing a coronary artery under constant pressure with blood at different temperatures. They have showed that cooling and warming the blood perfusing a coronary artery increases and decreases, respectively, the blood flow through this artery, the coronary vessels dilating on cooling and contracting on warming. However, if the temperature of the heart muscle itself decreases, the coronary flow tends to decrease, whereas an increase of the heart muscle temperature tends to increase the coronary flow. These latter changes are due to the fact that cooling of the heart muscle produces a more forceful and more prolonged heart beat which increases the systolic restriction of flow in the preparation used by Anrep and Häusler (9). These authors conclude that the direct effect of lowering the temperature on the coronary vessels, which tends to increase the coronary flow, is more pronounced than the indirect effect which tends to decrease the coronary flow by increasing the strength of the myocardial contraction. This conclusion has been confirmed by Nakagawa (133) who, measuring the coronary sinus outflow in the heart-lung preparation, has shown that cooling the blood from 38–40° to 28–30°C. increases the coronary flow. Further cooling seems to decrease the coronary flow.

Valvular lesions. As previously mentioned in the section on the effect of changes in cardiac output and work on the coronary circulation, acute pulmonary and aortic stenosis (84, 86) in the whole dog increases the coronary flow in the right and left coronary arteries, provided the aortic pressure remains fairly constant. Either lesion increases the work and the metabolism of the corresponding ventricle. No experimental data are available about the effect of mitral lesions on the coronary circulation. The effect of aortic insufficiency has not been adequately studied but it seems that aortic insufficiency increases the coronary flow during systole and lowers it during diastole. When aortic insufficiency is marked enough to lower the diastolic pressure markedly, the increase in coronary flow during systole does not compensate for the decrease in flow during diastole and the mean coronary flow decreases (71). In one patient with aortic insufficiency, the output and the work of the left ventricle have been found to be increased, as also were the oxygen consumption per 100 grams of left ventricle and the coronary flow per 100 grams of left ventricle (16).

Shock. With the demonstration that there can be an element of myocardial depression (179) in shock, it became imperative to find whether the myocardial depression of shock might not possibly be due to the probable decrease in coronary flow brought about by the decrease in blood pressure. Opdyke and Foreman (135) have shown that in hemorrhagic hypotension and shock the coronary flow decreases as the blood pressure falls but that the resistance to flow in the coronary bed is greatly reduced; this decrease in the resistance of the coronary bed has been interpreted as being due mainly to vasodilatation of the coronary bed. Following reinfusion of all withdrawn blood and restoration of mean aortic pressure to control level, the coronary flow increases markedly above its control value. This increase in coronary flow has also been interpreted as being due to coronary dilatation. Obviously concomitant determinations of coronary flow, cardiac work and metabolism throughout the period of hypotension and shock are necessary for a complete understanding of the mechanisms through which myocardial depression develops in shock.

Cardiac metabolism and efficiency. The state of nutrition of the myocardium depends not only upon the ratio between the volume of coronary blood flow and the amount of work performed by the heart, but also upon the metabolism and the efficiency of the heart. Although the effect of an increase in cardiac work on the coronary flow has been discussed previously, no systematic discussion has been presented of how a change in the amount of cardiac work affects the metabolism of the heart and whether physiological agents may affect the efficiency of the myocardium.

Evans and Matsuoka (48) have showed that in the heart-lung preparation an increase in the work of the heart produced by an increase in the arterial pressure or an increase of cardiac output leads to an increase in the oxygen consumption of the heart and an increase in its efficiency up to a certain level of work, beyond which the efficiency of the heart decreases. An increase in work due to an increase in output is performed by the heart more economically than an increase in work due to a rise in arterial blood pressure; therefore the heart is able to work better over wider ranges of output than of arterial pressure. Starling and Visscher (156) have demonstrated in the heart-lung preparation that the oxygen consumption of the heart, maintained under constant chemical and temperature conditions, is determined by its diastolic volume and, therefore, by the initial length of its muscular fibres and that during the whole of an experiment the oxygen consumption at a given diastolic volume is always the same, whatever the work the heart is performing at this volume. Since, in a heart in good condition, every increase or decrease in work is accompanied by a proportional increase or decrease in diastolic size, it follows that an increase in work demanded of the heart is met by a corresponding increase in the oxygen consumption of the heart, consequent on the increased initial length of its muscle fibres. As the heart tires, although the total energy as measured by oxygen consumption liberated at any given initial length of fibre remains unchanged, the amount of useful work performed decreases. In other words, to do the same amount of work the heart has to dilate continuously and the work is maintained constant at an ever-increasing cost in total energy.

These conclusions have been essentially confirmed by several investigators (66, 90, 96, 111). Starling and Visscher (156) have demonstrated that at the same diastolic length of fibre the heart uses more oxygen per beat when contracting at a low rate than at a high rate. However, slowing of the heart enables it to do a given amount of work per unit of time more economically; this has been corroborated by Cohn and Steele (24). These latter workers have also found that the oxygen consumption per gram of heart decreases with age (25). Gollwitzer-Meier and Krüger (69) have showed on the innervated heart-lung preparation that electrical or reflex stimulation of the vagi decreases the oxygen consumption of the heart and increases its efficiency whereas stimulation of the stellate ganglion increases the oxygen consumption of the heart and decreases its efficiency.

A number of studies has been done on the whole animal. Harrison, Friedman and Resnik (93) have confirmed that an increase in cardiac work brought about by increasing the cardiac output or raising the arterial blood pressure increases the coronary flow, the oxygen consumption of the heart and its mechanical efficiency. Gregg and Shipley (86) and Shipley and Gregg (148) have observed that an increase in cardiac work produced by artificially raising the pulmonary or the aortic pressure or by stimulating the stellate ganglia increases the oxygen consumption of the heart. Eckenhoff, Hafkenschiel, Foltz and Driver (34) have observed that, after the subdural injection of procaine hydrochloride or the intravenous injection of tetraethylammonium chloride, the fall in arterial blood pressure is associated with a diminished cardiac output and coronary flow; cardiac work, oxygen consumption and efficiency are reduced. Eckenhoff, Hafkenschiel, Landmesser and Harmel (37) have found that when a primary increase in mean arterial blood pressure is induced by a compression of the aorta severe enough to decrease the cardiac output, the cardiac work decreases, the coronary flow increases, the oxygen consumption of the heart rises and the cardiac efficiency decreases. When a primary increase in cardiac output is induced by the infusion of blood and/or isotonic gelatin in saline, blood pressure, coronary flow, cardiac work, oxygen consumption and efficiency rise. In anoxic anoxia, the cardiac output and work decrease, the coronary flow increases, the blood pressure and cardiac oxygen consumption remain constant and the cardiac efficiency falls. These authors have interpreted their findings as meaning that cardiac efficiency tends to vary directly with cardiac output and inversely with arterial blood pressure.

IV. PHARMACOLOGY

After a review of the methods used to study the coronary circulation and a review of the physiology of the coronary circulation, it will now be much easier to discuss the pharmacology of the coronary circulation. As a general pattern, it is planned first to present the available information on the effect of each drug on the whole animal; then, through an analysis of work done on more artificial preparations, an attempt will be made to elucidate the mechanisms of action of each drug by discussing its effect on the different determinants of the coronary flow. In the case of many drugs, such a program cannot be fulfilled because of lack of data. For convenience, the different drugs have been divided into three groups: (1) the drugs which are known for their vasopressor property; (2) the drugs known for their vasodepressor property; and (3) a group of miscellaneous drugs that are not especially known for their pressor or depressor effects after usual doses, although some of them exhibit pressor or depressor effects after higher doses.

Vasopressor drugs

Except under unusual circumstances of dosage and route of administration, in which case the vasopressor drugs may increase the coronary flow without modifying the mean arterial blood pressure, all the drugs discussed in this section increase both blood pressure and coronary flow. The mechanism of the increase in coronary flow is not entirely clear. Undoubtedly, the mechanical effect of an increase in the arterial blood pressure is responsible for at least part of the increase in flow. When the blood pressure rises, it is probable that the work of the heart increases and even when the dose of the drug is such that the arterial blood pressure does not rise it is probable that the work of the heart increases because of the increase in cardiac output (54). In either case, the increase in cardiac work is probably responsible for part of the increase in coronary flow (13, 84, 86). Whether all or some of the drugs presented in this section also have a direct effect on the coronary bed, independently of the increase in cardiac work, will be discussed when data are available, as will also be the effect of such drugs on the ratio $\frac{\text{cardiac work}}{\text{coronary flow}}$ and on the mechanical efficiency of the heart.

Epinephrine. In all the recent work, several groups of workers have shown that in the whole dog, anesthetized or unanesthetized, epinephrine always increases the coronary flow. Essex, Wégria, Herrick and Mann (47) have found that in the unanesthetized dog, doses of 0.002 to 0.1 mgm. of epinephrine given intravenously to dogs weighing from 12 to 20 kg. increase the blood flow in the left and in the right coronary arteries. The effect lasts from 1 to 4 minutes and only occasionally longer. In anesthetized dogs, weighing from 15 to 35 kg., Wégria, Essex, Herrick and Mann (175) have observed that doses of 0.025 to 0.050 mgm. of epinephrine injected intravenously markedly increase the coronary flow in both the right and the left coronary arteries and raise the mean arterial blood pressure. The heart rate is decreased during the early stage of the action of the drug. Blood pressure, heart rate and coronary flow return to control levels simultaneously, generally in 2 to 3 minutes, although the effect of the drug occasionally lasts longer. After reaching the control level, coronary flow and blood pressure occasionally fall and remain below the control level. Sometimes the blood pressure returns to, or falls below, the control value, while the blood flow in both coronary arteries is still increased. Green, Wégria and Boyer (74), using an orifice-meter in anesthetized dogs, have studied the effect of intravenous and intracoronary administration of commercial epinephrine solutions and of solutions of the pure drug. They have shown that the two types of preparation have essentially the same effect. The minimal intracoronary effective dose is 0.001 mgm. of epinephrine. Doses of epinephrine of 0.10 mgm. or less in dogs weighing an average of 15 kg. always increase coronary flow. The increase in coronary flow occurs simultaneously with the rise in blood pressure or a few seconds after signs of myocardial stimulation are present; with small doses of epinephrine given by the intracoronary route, there may be no increase in blood pressure but only an increase in the vigor of the myocardial contraction. Eckenhoff, Hafkenschiel and Landmesser (36), using the bubble flowmeter in anesthetized dogs weighing an average of 13 kg., have observed that the intracoronary injection of 0.00002 to 0.002 mgm. of commercial epinephrine increases the coronary flow. With the smaller doses, a definite increase in coronary flow occurs without any significant change in blood pressure or heart rate whereas higher doses increase both blood pressure and coronary flow. Doses of 0.050 to 0.5 mgm. of epinephrine given intravenously have an effect similar to that reported above (74, 175) and increase both blood pressure and coronary flow. The observations of Shipley and Kohlstaedt (150) have essentially confirmed the work previously mentioned. However, in their experiments, when small doses of epinephrine (0.0002 to 0.00002 mgm.) are injected into a coronary artery, first a definite increase in coronary flow occurs without any change in blood pressure or heart rate, and then the blood pressure rises and the coronary flow increases further.

It seems that there is little doubt that the effect of epinephrine in minimally effective as well as in larger doses is to increase the coronary flow in the whole dog, anesthetized and unanesthetized. When epinephrine raises the arterial blood pressure, the increase in arterial blood pressure undoubtedly accounts for at least some of the increase in coronary flow. It is also probable that the doses of epinephrine studied in the papers mentioned above increase cardiac output and work, as has been observed recently by Frank, Misrahy, Sioussat, Sommer, McCormack and Wégria (54), which would be another cause of the increase in coronary flow. Whether or not epinephrine, besides increasing coronary flow by increasing blood pressure, cardiac output and cardiac work, also induces a dilatation of the coronary bed by direct action has been a moot question for many years. In the experiments mentioned above, Wégria, Essex, Herrick and Mann (175) have calculated the changes in the resistance of the coronary bed produced by epinephrine from the formula, resistance $= \frac{\text{pressure}}{\text{flow}}$ and found that epinephrine decreases the resistance of the coronary bed. Whether such a simple approach to a complex problem is valid remains to be established.

However, Green, Wégria and Boyer (74) have arrived at the same conclusion by a more reliable approach. They have determined the ratio of the instantaneous rate of flow to the aortic pressure at the very end of diastole, a time of the cardiac cycle at which the extravascular support is more likely to be minimal and to remain constant under different hemodynamic conditions. This ratio, which they have called conductance, is markedly increased by the administration of epinephrine, especially when the drug is injected directly into a coronary artery. They have also shown that, during the rise of aortic blood pressure due to epinephrine, the ratio $\frac{\text{mean coronary flow}}{\text{mean aortic pressure}}$ is increased, the coronary flow increasing relatively more than the blood pressure, whereas an equal increase in aortic pressure produced by compression of the aorta decreases the ratio $\frac{\text{mean coronary flow}}{\text{mean aortic pressure}}$, the coronary flow rising relatively less than the aortic pressure. The rise in the ratio $\frac{\text{mean coronary flow}}{\text{mean aortic pressure}}$ is especially marked when epinephrine is injected directly into a coronary artery. Green, Wégria and Boyer (74) have concluded that epinephrine increases the coronary flow by increasing the blood pressure and possibly the cardiac output, but also that epinephrine decreases the resistance of the coronary bed. Because epinephrine increases the ratio $\frac{\text{flow}}{\text{pressure}}$ at the end of diastole, Green, Wégria and Boyer (74) have thought it legitimate to conclude that the decrease in the resistance of the coronary bed is due to dilatation of the coronary bed and not to a decrease in the magnitude of the extravascular support; this conclusion, it must be said, is probably correct but far from definitely proven. Eckenhoff, Hafkenschiel and Landmesser (36) have also reached the conclusion that epinephrine dilates the coronary bed since small intracoronary doses increase the coronary flow without affecting heart rate or arterial blood pressure. In their experiments, however, they did not rule out the possibility of changes in the magnitude of the extravascular support as a cause of the decrease in the resistance of the coronary bed; this possibility, it is granted, is unlikely. Shipley and Kohlstaedt (150) have confirmed the findings of Eckenhoff, Hafkenschiel and Landmesser (36).

As can be seen, the several groups of workers who have been interested in the effect of epinephrine on the coronary circulation are agreed that part of the effect of epinephrine on the coronary circulation is to decrease the resistance of the coronary bed, probably by producing dilatation of the coronary bed. The mechanism of this vasodilatation is more difficult to determine. It is agreed that when epinephrine is given in doses that increase the cardiac work by raising the blood pressure and/or increasing the cardiac output, coronary vasodilatation is probably due at least in part to the increase in cardiac load and metabolism, as has been discussed previously. However, since small doses of epinephrine injected directly into the coronary artery increase the coronary flow without modifying the blood pressure (36, 150), it seems possible that epinephrine produces vasodilatation by acting directly on adrenergic receptors of smooth muscle cells in the wall of the coronary vessels. Green, Wégria and Boyer (74), however, never observed an increase in the coronary flow after the intracoronary administration of epinephrine which occurred before the rise in blood pressure or the increase in vigor of the ventricular contraction. On the contrary, the increase in flow begins several seconds after the first manifestations of myocardial stimulation, and since the above authors were recording phasic changes in coronary flow they were in a better position than the other groups (36, 150) to detect early manifestations of cardiac stimulation. Therefore it is felt that, if epinephrine dilates the coronary bed by a direct action on the coronary vessels, it has not been demonstrated in the work that has just been reviewed.

The amount of research on the effect of epinephrine on the coronary circulation is tremendous and reference will be made only to those investigations which add something new to what has already been said about epinephrine from experiments performed on the whole animal with modern technics. Space does not permit mention of those studies performed with old technics or in highly artificial preparations, except those that throw some additional light on the mechanism of action of the drug.* An elegant attempt to determine whether epinephrine has a direct vasodilating effect on the coronary bed has been made by Katz, Lindner, Weinstein, Abramson and Jochim (108) who have studied the effect of epinephrine in cats and dogs by perfusing the coronary bed of the isolated heart in ventricular fibrillation under constant pressure with defibrinated blood. This preparation has been discussed in the section on methods. In the cat heart, epinephrine decreased the coronary flow in four experiments and increased it in eight experiments. In the dog heart, epinephrine either increased the coronary flow or increased it after having decreased it momentarily. No correlation was found between dose or concentration of the drug on the one hand and the nature and magnitude of the response on the other hand. This work is of great interest because it brings out the fact that different species may possibly react differently to the same drug. Other workers (92, 125) have concluded from their experiments that epinephrine dilates the coronary bed, probably by a direct action on the blood vessels.

In short, epinephrine increases the coronary flow in the whole animal. This increase in flow is due to an increase in cardiac load produced by an increase in cardiac output and/or a rise in blood pressure, which leads to a decrease in the resistance of the coronary bed, probably due to coronary vasodilatation. Epinephrine also seems to dilate the coronary vessels by acting directly on the coronary wall, although in certain species epinephrine may produce constriction of the coronary bed. There is little information available on the effect of epinephrine on the ratio $\frac{\text{coronary flow}}{\text{cardiac work}}$ and on the efficiency of the heart. In the denervated heart-lung preparation, epinephrine increases coronary flow and oxygen consumption of the heart but decreases efficiency of the heart. There is a short initial period during which the work of the heart is increased and the oxygen consumption is unchanged, which indicates an increase in the efficiency of the heart (67). In the innervated heart-lung preparation and in the whole animal (68), the reflex effects of epinephrine may counterbalance or even offset the direct effects of epinephrine on the myocardium and the coronary circulation.

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* For supplementary bibliography, see references 70, 74, 102, 108, 140, 151 and 175.

It is pertinent to mention that Levine, Ernstene and Jacobson (119) have been able to induce anginal pain in patients with coronary sclerosis by the subcutaneous injection of epinephrine. Unless it is postulated that epinephrine induces a constriction of the coronary artery, it must be accepted that at least in persons with coronary sclerosis the drug decreases the efficiency of the heart and/or increases the work and the metabolism of the heart more than the coronary flow. If this view is correct, it does not follow that epinephrine has the same effect in normal individuals in whom the coronary vessels may be able to accommodate an amount of coronary flow commensurate with the increase in cardiac work and metabolism.

Nor-epinephrine. Recently, Wégria, Ward, Frank, Dreyfuss, Brown and Hutchinson (183) have studied the effect of *l*-nor-epinephrine on arterial blood pressure and coronary blood flow in the anesthetized dog and found that nor-epinephrine is essentially similar in its action to epinephrine, although no systematic comparative study was carried out. Given intravenously in doses of 0.002 mgm. to 0.030 mgm. per kg., it increases coronary flow and arterial blood pressure. Its effect lasts from 2 to 6 minutes. No attempt was made to calculate the changes in the resistance of the coronary bed during the period of action of the drug and it is not known whether the increase in coronary flow is entirely due to the increase in the aortic pressure or whether the drug also modifies the resistance of the coronary bed. During that part of the period of action of the drug when either blood pressure or coronary flow has returned to the control level, no consistent effect on the coronary resistance is observed. The effect of the drug is the same in dogs in good condition and in dogs whose circulatory condition has deteriorated, as judged by the level of their blood pressure.

Ephedrine. Stoland and Ginsberg (159) have observed that the intravenous injection of 2.5 to 10 mgm. of ephedrine in dogs weighing between 8 and 13 kg. increases the arterial blood pressure and the coronary sinus outflow. The maximal increase in coronary flow occurs after the blood pressure has returned to control level. Some increase in flow sometimes persists for 30 to 90 minutes or longer. Obviously the increase in the coronary flow cannot all be explained by an increase in the aortic pressure, since in the experiments described above there is a time when the coronary flow is still increased but the blood pressure has returned to or even fallen below the control level. It cannot be stated whether this obvious decrease in the resistance of the coronary bed is due to a direct effect of the drug on coronary vessels, to an increase in the work of the heart, the cardiac output being still increased when the blood pressure is back to the control level, or to the lasting effect of a relative myocardial ischemia that develops during the hypertensive stage of the action of the drug. Leyko (121) has reported that ephedrine and ephetonine increase the coronary sinus outflow in the dog heart-lung preparation in which arterial blood pressure and cardiac output are constant. Since in Leyko's experiments the volume of the heart decreased with ephedrine and ephetonine, it would seem that these drugs increase the coronary flow not by decreasing the extravascular support but by dilating the coronary bed by acting directly on the blood vessel walls. Narayana (134) has essentially confirmed Leyko's findings.

Paredrine. Paredrine hydrobromide and paredrinol sulfate have been studied by Elek and Katz (39) and found to have the same action. Using the perfusion of the dog heart in ventricular fibrillation under constant pressure as previously described (108), Elek and Katz have observed that doses of 0.5 to 1.5 mgm. of paredrine have no effect but that doses of 2 mgm. of paredrine cause a prompt and marked increase of the coronary flow lasting from one-half to one hour. The increase in coronary flow was interpreted as due to coronary dilatation. It is probable that the effect of this drug on the coronary flow of the whole animal is qualitatively similar to that of epinephrine and ephedrine, although to our knowledge the effect of this drug on the coronary flow of the whole animal has not been investigated.

Nicotine. Kountz (114) has studied the effect of nicotine on the coronary flow of the revived human heart, perfused under constant pressure according to the Langendorff method, and found that nicotine decreases the coronary flow and increases the rate and the amplitude of contraction of the ventricles. It also either constricts rings of human coronary arteries or has no effect on them (114). However, in the whole animal nicotine increases blood pressure and coronary flow, its effect being very similar to that of epinephrine (174).

To summarize our knowledge of the vasopressor drugs, it can be said that a great deal is known about epinephrine, much less about nor-epinephrine, ephedrine, paredrine and nicotine. About other similar drugs, nothing at all is known as to their effects on the coronary circulation. Studies of their effect simultaneously on blood pressure, coronary flow and cardiac output, work, metabolism and efficiency are needed. From what is known, it seems that all the drugs considered above have qualitatively a similar type of effect.

Vasodepressor drugs

In this section will be reviewed those drugs that are conspicuous for being vasodilator and, if given in sufficient dose, hypotensive. A few preliminary considerations that apply to the group as a whole will ultimately save time and space.

All these drugs have in common the capacity to increase the coronary flow even when a dose large enough to decrease the blood pressure, within reason, has been given. The "within reason" cannot be expressed precisely, may vary with different drugs and seems to depend on factors such as the type of anesthesia, as has been pointed out previously, and the condition of the animal. These drugs differ in intensity and duration of action and it is always hazardous to make any statement about the former when the animals on which the drugs are tested have been subjected to anesthetics and extensive operative procedures.

These drugs may also differ in the mechanisms by which they produce systemic vasodilatation and in the fact that some have a direct effect on the myocardium and others do not. As has been said, all these drugs increase the coronary flow in the whole animal unless the decrease of blood pressure is too marked. The increase in the coronary flow is due to a decrease in the resistance of the coronary bed. With the data available, it is often difficult to ascertain whether the decrease in the resistance of the coronary bed is due to vasodilatation or to a decrease in the magnitude of the extravascular support. It is equally difficult to determine whether the dilatation of the coronary bed, if such is proven, is due to a direct effect of the drug on the coronary vessel wall or whether it is caused by an indirect effect of the drug on the coronary bed via an effect of the drug on cardiac output, work and metabolism; in turn this effect on cardiac output, work and metabolism may be the result of a direct action on the myocardium or an indirect result of the hypotensive action (23, 181). It must also be remembered that hypotension *per se* reflexly affects the coronary circulation (13, 158) by way of the carotid sinus. Thus it is obvious that the simultaneous effects of each of these drugs on blood pressure, coronary flow, and cardiac output, work, metabolism and efficiency should be studied.

Papaverine. Papaverine increases the coronary flow of the unanesthetized (47) and anesthetized (175) dog. Doses of 15–20 mgm. given intravenously to dogs weighing from 12 to 15 kg. decrease blood pressure and increase heart rate and coronary flow. These observations have been confirmed by Eckenhoff and Hafkenschiel (33). The increase in coronary flow is marked and lasts from 2 to 5 minutes. Lindner and Katz (122) have observed an increase in the coronary flow following papaverine in the perfused dog heart in ventricular fibrillation. It remains for future investigation to determine how much of the increase in the coronary flow in the whole animal is due to the direct effect of papaverine on the coronary bed and how much is due to other factors, especially a possible increase in the work and the metabolism of the heart. It is of interest that Russek, Smith, Baum, Naegele and Regan (145) have reported that, in a patient with coronary sclerosis, papaverine given intravenously or orally can prevent to a great extent the anginal pain and the electrocardiographic changes pathognomonic of myocardial anoxia induced by a standard exercise tolerance test.

Nitrites. In the unanesthetized dog amyl nitrite and nitroglycerin increase the coronary flow (41, 47). The intravenous administration of 0.22 mgm. to 1.3 mgm. of nitroglycerin to dogs weighing around 15 kg. or the inhalation of amyl nitrite increases the coronary flow for 1 to 3 minutes. When similar doses of nitroglycerin are given by mouth, the increase in flow persists for longer than 16 minutes (47). In the anesthetized dog, Boyer and Green (20) have showed with the orifice-meter that the intracoronary or intravenous injection of nitroglycerin or sodium nitrite lowers blood pressure and increases coronary flow and heart rate; they conclude that nitroglycerin and sodium nitrite decrease the resistance of the coronary bed by inducing directly a dilatation of the coronary vessels. Whether changes in cardiac work also play a rôle cannot be determined in their experiments. In the anesthetized dog, Wégria, Essex, Herrick and Mann (175) have observed that amyl nitrite by inhalation increases the coronary flow in both right and circumflex coronary arteries, increases the heart rate and lowers the mean arterial blood pressure; the increase in flow lasts 1 to 6 minutes. The intravenous injection of nitroglycerin has essentially the same effects. Eckenhoff and Hafkenschiel (33), using a bubble flowmeter in anesthetized dogs, have showed that the smallest effective dose of nitroglycerin (0.002 to 0.02 mgm.) given by intracoronary injection increases the coronary flow as long as the mean arterial blood pressure does not fall too much. A larger dose, 0.2 mgm., increases the coronary flow until the drug reaches the systemic circulation at which time, if the arterial blood pressure falls markedly, the coronary flow decreases. Essentially similar effects result from the intramuscular, intravenous, subcutaneous and sublingual administration of the drug. Amyl nitrite (33) also lowers arterial blood pressure and increases heart rate and coronary flow. Katz, Lindner, Weinstein, Abramson and Jochim (108) have shown that sodium nitrite and nitroglycerin dilate the coronary vessels of the perfused isolated dog heart in ventricular fibrillation. Bodo (18) has observed in the dog heart-lung preparation that both amyl nitrite and sodium nitrite increase coronary flow while arterial blood pressure and cardiac output remain constant; but, whereas amyl nitrite does not modify the size of the heart, sodium nitrite, at least in large doses, causes the heart to dilate. Bodo's experiments show, of course, that the nitrites can decrease the resistance of the coronary bed by dilating the coronary vessels directly, but it is difficult in experiments in which the heart size is altered to draw any definite conclusion as to the rôle played by the probable changes in the magnitude of the extravascular support. It is of interest that Levy, Bruenn and Williams (120) have showed that, in patients with coronary sclerosis, nitroglycerin delays the appearance of the anginal pain and diminishes the intensity of the electrocardiographic signs pathognomonic of the cardiac anoxia induced by an anoxemia test. This has been confirmed in one patient by Russek, Smith, Baum, Naegele and Regan (145) by the use of a standard exercise tolerance test.

Nucleic acid derivatives. Drury and Szent-Györgyi (32) have shown that the coronary sinus outflow in a dog heart-lung preparation is increased by adenosine and adenylic acid, a finding which has been confirmed by Wedd (167) on the perfused rabbit heart. Wedd and Drury (169), measuring the coronary sinus outflow with a Morawitz-Zahn cannula in the anesthetized dog, have shown that adenosine, muscle and yeast adenylic acid and yeast cytidylic acid given intravenously increase the coronary flow while the arterial blood pressure is unchanged or lowered; the increase in flow lasts about 3 minutes. Essex, Wégria, Herrick and Mann (47) have observed an increase in the coronary flow of the unanesthetized dog given muscle adenosine phosphoric acid. Recently Eckstein, Chambliss, Demming and Wells (38) have reported that a product liberated in the mechanical destruction of red cells, probably ADP or ATP, is a potent coronary dilator.

Histamine. Doses of 0.1 to 0.5 mgm. histamine acid phosphate given intravenously to unanesthetized dogs weighing around 20 kg. increase the coronary flow (47) for 2 to 3 minutes. In the anesthetized dog (175), the same effect is observed. The curves of the effects of histamine on blood pressure, heart rate and coronary flow are essentially the same as those of papaverine and nitroglycerin. Katz, Lindner, Weinstein, Abramson and Jochim (108) have shown that histamine dilates the coronary vessels of the perfused fibrillating dog ventricles. The effect of histamine on other preparations is generally to increase the coronary flow but it is sometimes difficult from these preparations to derive any new information as to the mechanisms of action of the drug in the whole animal. For example, histamine increases the coronary flow in the revived beating human heart probably by dilating the coronary bed; but, in the revived human heart arrested by perfusion with solutions of high pH, histamine has a variable effect, and when the heart has been arrested by perfusion with solutions of low pH histamine has no effect or increases the coronary flow slightly (116). Narayana (134) has shown conclusively that histamine increases the coronary flow in the dog heart-lung preparation. As suggested by Gunn (91), there may be a species difference in the response of the coronary vessels to histamine as he has observed that histamine increases the coronary outflow of the perfused cat heart but decreases it in the rabbit heart, the effect on flow in either case being probably due mainly to a direct effect of the drug on the coronary bed.

Procaine. Wégria, Ward, Frank, Dreyfuss, Brown and Hutchinson (183) have recently shown that the intravenous administration of 1 to 10 mgm. of procaine hydrochloride per kg. to anesthetized dogs has essentially the effect of the hypotensive drugs; it produces an immediate and temporary decrease in blood pressure and coronary flow, after which both increase. The coronary flow comes back to the control level and in most cases rises above it, at which time the blood pressure is still below or at the control level. The transient decrease in coronary flow and blood pressure may be absent. During most of its period of action, procaine obviously decreases the resistance of the coronary bed. The effect of the drug lasts from 2 or 3 minutes to more than 23 minutes. Eckenhoff, Hafkenschiel, Foltz and Driver (34) have observed that in the anesthetized dog the subdural administration of a dose of procaine sufficient to lower the arterial blood pressure and reduce the cardiac output and work, decreases the coronary flow, the cardiac oxygen consumption and the efficiency of the heart.

Khellin. The effect of khellin on the coronary circulation has recently aroused a great deal of interest. Anrep, Barsoum, Kenawy and Misrahy (3) were the first to study the effect of khellin on the coronary circulation experimentally and clinically. They have shown that in the dog heart-lung preparation 10 mgm. of khellin increases the coronary sinus outflow very markedly and this increase remains maximal for over 3 hours. Concentrations of khellin as low as 1:2,000,000 are effective. Since the size of the heart does not change, it seems likely that khellin increases the coronary flow in the denervated heart-lung preparation by dilating the coronary vessels. In the anesthetized dog, rapid intravenous injections of 20 to 30 mgm. of khellin lower the heart rate and the blood pressure for 1 or 2 minutes, but slow intravenous injections of khellin at the rate of 2 mgm. per 30 seconds can be continued for a long time without such effects. In the whole anesthetized dog, 1 mgm. per kg. of khellin does not lower the blood pressure but increases the coronary flow markedly for more than one hour. It is probable that in the whole animal, other mechanisms than the direct effect of the drug on the coronary vessels play a rôle. Such mechanisms have been mentioned in the introduction to the discussion of the vasodepressor drugs. Killam and Fellows (112), Fellows, Killam, Toner, Dailey and Macko (50) and Wégria, Ward, Dreyfuss, Brown and Hutchinson (183) have essentially confirmed these observations on the anesthetized dog. Khellin has also been reported to prevent and to relieve the anginal pain and to prevent the appearance of pain and electrocardiographic changes induced by exercise in a standard exercise tolerance test (3, 10, 30, 143). Recently much less favorable results have been reported by one group of investigators (89).

Tetraethylammonium. In the anesthetized dog, it has been shown with the rotameter that 0.125 to 2.0 mgm. doses of tetraethylammonium chloride per kg. given intravenously decrease blood pressure, coronary flow and heart rate (183). During the period of recovery, a decrease in the resistance of the coronary bed is seen in some experiments and an increase in others. Eckenhoff, Hafkenschiel, Foltz and Driver (34) have observed that the intravenous injection of 2 or 4 mgm. of tetraethylammonium chloride per kg. in dogs lowers arterial blood pressure and decreases coronary flow as well as cardiac output, work, oxygen consumption and efficiency.

Xanthines. Essex, Wégria, Herrick and Mann (47), using the thermostromuhr in the unanesthetized dog, have shown that the intravenous administration of 120 to 480 mgm. of theophylline ethylenediamine (aminophylline) to dogs weighing from 7.5 to 20 kg. increases the coronary flow for 2 to 24 minutes or longer. Wégria, Essex, Herrick and Mann (175) have observed that doses of 240 to 480 mgm. of aminophylline given intravenously to dogs weighing 18 to 35 kg. and anesthetized with chloralosane increase the heart rate, lower the blood pressure and increase the coronary flow in both right and circumflex coronary arteries. In some cases, the flow is still increased after 28 minutes. In short, the effect of these doses of aminophylline on heart rate, coronary flow and arterial blood pressure is essentially similar to that of other vasodepressor drugs. The effect of aminophylline generally lasts longer than that of the other vasodepressor drugs discussed above, except khellin. Since in these experiments the coronary flow is increased while the arterial blood pressure is lowered or unchanged, aminophylline increases the coronary flow by decreasing the resistance of the coronary bed. Green (70) and Boyer and Green (20), studying the effect of the intracoronary and intravenous administration of theophylline, theobromine, aminophylline and theophylline monoethanolamine (theamine), have confirmed that the xanthines increase the coronary flow by decreasing the resistance of the coronary bed, this decrease probably being brought about by dilatation of the coronary bed. However, Boyer and Green (20) mention that the tracing of the coronary flow recorded with an orifice-meter affords evidence of a stimulating effect of the xanthines on the myocardium; whether the dilatation of the coronary bed is due to the effect of changes in the work of the heart on the coronary bed or to a direct effect of the drug on the coronary vessels or both remains to be determined. Eckenhoff and Hafkenschiel (33) have observed with the bubble flowmeter that in the anesthetized dog the intracoronary injection of 1 mgm. of aminophylline increases the coronary flow and raises the arterial blood pressure slightly. The intravenous administration of 60 to 120 mgm. of aminophylline has the same effect as that reported above (175); it lowers the arterial blood pressure and increases coronary flow and heart rate. Essentially similar results have been observed by Stoland, Ginsberg, Loy and Hiebert (161) who measured the effect of aminophylline on the coronary sinus outflow in the dog denervated heart-lung preparation and in the anesthetized dog. Gilbert and Fenn (55), measuring the coronary sinus outflow with a Morawitz-Zahn cannula, have confirmed that the xanthines, given intravenously in doses similar to those used in humans, increase the coronary flow. Recently Foltz, Rubin, Steiger and Gazes (52) have studied the effect of the intravenous administration of 4 mgm. of aminophylline per kg. on the coronary circulation of anesthetized dogs. Since the method used to determine the coronary flow was the nitrous oxide method, a period of 30 minutes has to be allowed between the control determinations and the measurements after administration of the drug. This period of 30 minutes allows the animal to eliminate the nitrous oxide inhaled during the first determination. The second set of determinations is obtained after the blood pressure has stabilized following the injection of aminophylline. Because control experiments of similar duration, during which no aminophylline is injected, show a great deal of spontaneous variation in the different cardiac functions measured, it is difficult to assess the effect of aminophylline from this work, as the authors themselves point out. It seems, however, that under the experimental conditions mentioned, aminophylline increased the heart rate, the cardiac output and the work of the heart; the arterial blood pressure was unchanged or lowered at the time of the measurement; the changes in coronary flow and cardiac oxygen consumption were of no statistical significance; the coronary oxygen arteriovenous difference was increased in two experiments and essentially unchanged in the other two. Realizing the shortcomings of this work, Foltz, Rubin, Steiger and Gazes (52) have followed the effect of the doses of aminophylline used in the work mentioned above, on the coronary oxygen arteriovenous difference in experiments of shorter duration without measuring the coronary flow. They have demonstrated that aminophylline consistently decreases the oxygen content of the coronary sinus venous blood and increases the coronary oxygen arteriovenous difference at the peak of the effect of the drug, which is of interest but difficult to interpret decisively without concomitant measurement of the coronary flow. These latter results also cast doubt on the validity of the first part of the work in which two out of four experiments failed to show this increase in the coronary oxygen arteriovenous difference.

Numerous papers deal with the effect of the xanthines on the perfused isolated heart of different species and all authors are agreed that the xanthines increase the coronary flow in such preparations. However, in general, little decisive information about the mechanism of action of the xanthines is gained from such experiments. Heathcote (95) has found that caffeine, theophylline and theobromine, in order of increasing potency, all augment the coronary flow in the perfused rabbit heart. Smith, Miller and Graber (152) have shown in the perfused rabbit heart that caffeine-sodium benzoate and theobromine sodium salicylate in 1:25,000 concentration have little or no effect on the coronary flow, whereas theophylline and aminophylline in similar concentration increase the coronary flow probably by dilating the coronary bed. Kountz (114) has observed that caffeine-sodium benzoate increases the rate and the strength of the ventricular contraction as well as the coronary flow in revived human hearts perfused by the Langendorff method. Kountz and Smith (115) have also found that the xanthines increase the coronary flow in the revived human heart, dilated as well as not dilated, in which the coronary bed is perfused under constant pressure. Lindner and Katz (122), using the perfused dog heart in ventricular fibrillation, have shown that aminophylline and caffeine-sodium benzoate dilate the coronary vessels, caffeine being less effective than aminophylline with respect to duration and intensity of effect. The concensus therefore seems to be that the xanthines increase the coronary flow in the whole animal by dilating the coronary bed through a direct action on the coronary vessels but other mechanisms mentioned in the discussion of the vasodepressor drugs also play a part. A study of the effect of the xanthines simultaneously on coronary flow and cardiac output, work and efficiency is highly desirable.

Gold, Kwit and Otto (63) have concluded from careful clinical observations that theobromine and aminophylline, given orally for one to 25 weeks, influence neither the capacity for work nor the frequency or the severity of attacks of angina. This has been confirmed by Boyer (19) who, in a review of the subject, concludes that clinical evaluation of the usefulness of the xanthines in the treatment of coronary artery disease is far from satisfactory and that it seems wise to place the burden of proof on those who claim therapeutic efficacy for the xanthines in anginal pain due to coronary sclerosis. Fowler, Hurevitz and Smith (53) have reported that aminophylline promotes the development of the collateral circulation in experimentally induced cardiac infarction in the dog. Unfortunately neither Wiggers and Green (188) nor Gold, Travell and Modell (65) have been able to confirm these findings. It must be remembered, however, that Levy, Bruenn and Williams (120) have reported that, in patients suffering from angina due to coronary sclerosis, aminophylline in doses of 0.48 gram given intravenously causes a prolongation of 63 per cent in the time of appearance of pain and decreases the electrocardiographic manifestations of cardiac anoxia in anoxemia tests. Aminophylline taken orally has the same effect but it is less marked. It may well be that in attempts to evaluate the clinical usefulness of the xanthines the importance of the level of the drug in the blood has been neglected (165).

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Acetylcholine, acetyl-beta-methylcholine (methacholine), carbamylcholine (carbachol). Essex, Wégria, Herrick and Mann (47), using the thermostromuhr on the unanesthetized dog, have observed that 0.2 mgm. of methacholine given intravenously to a 13 kg. dog increased the coronary flow for 3 minutes. In the anesthetized dog (175) similar doses first increase and then decrease the flow in both the circumflex and the right coronary arteries, but after about 30 seconds the flow increases and remains elevated for 4 minutes or longer. The heart rate is increased throughout the period of action and the blood pressure falls very markedly, reaching a minimum within one minute after the injection; it then rises progressively toward the control level. Eckenhoff, Hafkenschiel and Landmesser (36), using the bubble-flowmeter in the anesthetized dog, have reported that 0.02 to 2.0 micrograms injected into a coronary artery produce an immediate and marked acceleration of flow lasting 1 to 3 minutes, without necessarily inducing any change in blood pressure or heart rate. Larger doses usually elicit a considerable increase in flow followed by a transitory asystole and decrease in flow. After atropine, acetylcholine is without effect. When given intravenously into the systemic circulation, acetylcholine always produces hypotension and a decrease in coronary flow. Anrep (2) reports that acetylcholine in small and large doses always increases the coronary flow in the dog heart-lung preparation as well as in the whole animal, and the effect is not abolished by atropine. Narayana (134) has confirmed that acetylcholine in doses of 6 mgm. increases the coronary flow in the dog heart-lung preparation. Katz, Lindner, Weinstein, Abramson and Jochim (108) have studied the effect of acetylcholine and methacholine on the coronary flow of the perfused isolated dog and cat heart in ventricular fibrillation. In the cat, concentrations of acetylcholine stronger than 1:820,000 cause vasoconstriction only, but with weaker concentrations vasoconstriction or vasodilatation or a diphasic reaction results. Methacholine to be effective has to reach a concentration stronger than 1:250,000 and then produces constriction. In the dog, acetylcholine and methacholine produce coronary dilatation only. Whatever the effect of the two drugs, it lasts from 2 to 4 minutes. Atropine abolishes or diminishes the effect of both drugs in the cat and the dog. Wedd (168) has studied the effect of acetylcholine, methacholine and carbachol on the coronary sinus outflow in the perfused beating heart of cats and rabbits as well as in the whole anesthetized dog, measured with a Morawitz-Zahn cannula. The results observed seem to be qualitatively the same with the three drugs but are quite variable in different experiments, even in the same species. However, the first intravenous injection of any one of the three drugs in the dog induces an increase in the coronary sinus outflow despite a decrease in arterial blood pressure, which confirms most of the previous work.

It is obvious that the effect of the choline derivatives on the coronary circulation deserves further study. At the present time it seems to be the consensus that during most of their period of action the choline derivatives increase the coronary flow in the whole dog unless the arterial blood pressure decreases too markedly. It seems that, at least in the dog, part of this increase in coronary

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MISCELLANEOUS DRUGS

In this section will be presented the drugs which do not conspicuously raise or lower the arterial blood pressure. Some of these drugs, however, have a definite effect on the blood pressure, but they are classified as miscellaneous drugs and not as vasodepressor or vasodepressor agents because of other pharmacological actions which set them aside from the drugs previously discussed.

Thyroxin. Essex, Herrick, Baldes and Mann (41) using the thermostromuhr have shown that 1 mgm. of thyroxin per kg. given intravenously increases the coronary flow of the unanesthetized dog within 48 hours. A maximal increase is reached within 48 to 96 hours, then the coronary flow decreases progressivelye Bing and his coworkers (16) have studied one patient with hyperthyroidism. and slight anemia and found that cardiac output, work of the left ventricle and coronary flow per 100 grams of left ventricle were definitely higher than in a group of normal human beings.

Angiotonin and renin. Hill and Andrus (98) have shown that renin does not alter the coronary flow but that angiotonin decreases it in the isolated cat heart perfused with Ringer-Locke solution by the Langendorff method. The decrease caused by angiotonin is frequently followed by a slight rise. The decrease in flow is probably not due to a change in amplitude of ventricular contraction since the decrease in flow is also observed when the ventricles are kept fibrillating. Lorber (123) has observed in the isolated cat and dog heart that angiotonin produces a decrease in coronary flow accompanied by a decrease in diastolic size of the heart and an increase in oxygen consumption, work and efficiency of the heart. Because of the decrease in diastolic size of the heart, it is impossible to be certain that the decrease in coronary flow might not have been caused at least partly by an increase in extravascular support. Elek and Katz (39) have observed that renin and angiotonin have variable effects on the coronary flow of the perfused dog heart in ventricular fibrillation; both renin and angiotonin decrease coronary flow in some preparations, increase it in others and sometimes are without effect, although both more often increase coronary flow.

Pentobarbital. Using the thermostromuhr, Essex, Wégria, Herrick and Mann (47) have shown in one experiment that the intravenous administration of 300 mgm. of pentobarbital sodium to a 19.5 kg. unanesthetized dog increased the coronary flow markedly. The flow was still definitely above the control value 82 minutes after administration of the drug.

Glucose. Ginsberg, Stoland and Loy (60) studied the effect of dextrose, given as a 50 per cent solution, on the coronary circulation. Using a modified Morawitz-Zahn cannula to measure coronary sinus outflow in the whole dog, they found that 5 grams of glucose injected in 2 minutes increased the coronary flow; the maximal increase occurred within 2 to 41 minutes and some increase persisted for 40 minutes or longer; the arterial blood pressure rose slightly but occasionally it decreased. When a second injection was given, it produced as great an increase in coronary flow or a greater increase than the first injection. The injection of 10 grams produced a greater increase than 5 grams. Similar amounts of glucose had no effect, slightly increased or slightly decreased the coronary flow of the denervated and innervated dog heart-lung preparation in which cardiac output and arterial pressure were presumably kept constant. The slight and very transitory decrease in the viscosity of the blood produced by the injection of the solution of glucose in the whole animal does not account for the marked and prolonged increase in coronary flow. It seems probable that the increase in output, work and metabolism of the heart due to the increase in the blood volume of the whole animal is responsible for the increased coronary flow. However, it must be added that in the whole animal a hypertonic solution of glucose increases the coronary flow more than does an equally hypertonic solution of sodium chloride. Ginsberg, Stoland and Loy (60) conclude that dextrose has no direct effect on the coronary circulation and that the main factor responsible for the increase of the coronary flow in the whole animal is an increase in blood volume and cardiac output, work and metabolism. Lindner and Katz (122) have shown in the perfused dog heart in ventricular fibrillation that dextrose in concentrations of 2 to 27 per cent dilates the coronary vessels although perfusion with large volumes of more concentrated solutions can stop the coronary flow.

Insulin. Smith has reviewed the effect of insulin and hypoglycemia on the heart (151). From a study of the effect of hypoglycemia and hyperinsulinemia upon the electrocardiogram of dogs, Soskin, Katz and Frisch (154) believe that insulin has a dual deleterious effect on the heart, one induced by hypoglycemia and another not antidoted by correcting or preventing the hypoglycemia. In an attempt to find out if insulin might possibly have a direct effect on the coronary vessels, Elek and Katz (39) have studied its effect on the coronary bed of the perfused dog heart in ventricular fibrillation and observed that it produces a marked coronary dilatation. They concluded that the deleterious effect of insulin must be due to its direct action on the myocardium. It must be added that besides the likely direct effect of insulin on the myocardium, insulin also increases the work of the heart when it induces hypoglycemia (40). Bodo (18), on the other hand, has observed that in the dog denervated heart-lung preparation insulin in small doses (5 units) has little or no effect on the coronary flow and in doses of 10 units always decreases coronary flow, the blood pressure, cardiac output and work remaining constant. However, since the volume of the heart decreases, it may well be that the decrease in flow is due to an increase in magnitude of extravascular support.

Camphor. Bodo (18), measuring coronary sinus outflow in the dog heart-lung preparation, has reported that camphor increases coronary flow slightly; since the heart increases in size, although the cardiac work remains constant, it may well be that this increase in coronary flow is not due to coronary dilatation but to a decrease in extravascular support. Kountz (114) has confirmed Bodo's findings on the effect of the drug on coronary flow in the revived human heart perfused by the Langendorff technic.

Pentamethylenetetrazol (*pentylenetetrazol, metrazol*). Stoland and Ginsberg (160), measuring the sinus outflow with a Morawitz-Zahn cannula in the dog denervated heart-lung preparation and in the anesthetized dog, have found that in the heart-lung preparation 90 to 180 mgm. of metrazol increase the coronary flow but a rise in blood pressure which sometimes occurs may be responsible. However, in some experiments, the coronary flow rises whereas the blood pressure falls. It seems, therefore, that metrazol increases the coronary flow at least partly by decreasing the resistance of the coronary bed. In the whole animal, doses of 20 to 90 mgm. have practically no effect on either blood pressure or coronary flow. In contrast to Stoland and Ginsberg's results, Leyko (121) has reported that metrazol does not affect the coronary circulation in the dog heart-lung preparation over a wide range of doses. Lindner and Katz (122) have shown that doses of 100 mgm. of metrazol increase coronary flow in the perfused dog heart in ventricular fibrillation. The increase in flow is slight and lasts about 2 minutes.

N-N-Diethyl-3-pyridinecarboxamide (*coramine, nikethamide*). Stoland and Ginsberg (160) have observed that in the dog denervated heart-lung preparation 250 mgm. of nikethamide produce a marked increase in coronary flow even when a temporary fall in blood pressure occurs; doses of 50 mgm. produce a slight and temporary increase in flow. Since no mention is made of changes in the size of the heart, even if an unchanged cardiac output is presumed it is impossible to rule out changes in the extravascular support as a possible cause of the increase in coronary flow. The same authors have found that in the whole anesthetized dog 250 mgm. is the smallest dose that will change coronary sinus outflow. In dogs weighing between 8 and 15 kg., 250 to 500 mgm. of nikethamide given intravenously increase coronary flow markedly even when the fall in arterial blood pressure is not artificially compensated. Wégria, Essex, Herrick and Mann (175) have observed that intravenous administration of 250 to 500 mgm. of nikethamide to anesthetized dogs weighing around 20 kg. increases blood pressure and coronary flow after an initial and very brief decrease in blood pressure and coronary flow. The increase in coronary flow generally lasts longer than the rise in blood pressure. In some experiments, the coronary flow increases while the blood pressure remains unchanged. The increase in flow lasts from 2 to 14 minutes. Greene (77) has reported that 625 mgm. of nikethamide given intravenously to a dog weighing 11 kg. increased the coronary flow very markedly although the arterial blood pressure decreased. Similar effects of nikethamide on coronary flow have been observed in the unanesthetized dog (47). Eckenhoff and Hafkenschiel (33) have recently studied the effect of nikethamide on the coronary circulation of the anesthetized dog with the bubble flowmeter. They have observed that 5 mgm. injected into a coronary artery increased the coronary flow; the arterial blood pressure remained unchanged and the heart rate was increased. The intravenous administration of 125 to 750 mgm. of the drug did not produce any consistent change in coronary flow or any great change in heart rate or arterial blood pressure. It seems, however, from the average of the data of all the experiments, that the coronary flow generally increases slightly, the arterial blood pressure decreases slightly and measuring the sinus outflow with a Morawitz-Zahn cannula in the dog denervated heart-lung preparation and in the anesthetized dog, have found that in the heart-lung preparation 90 to 180 mgm. of metrazol increase the coronary flow but a rise in blood pressure which sometimes occurs may be responsible. However, in some experiments, the coronary flow rises whereas the blood pressure falls. It seems, therefore, that metrazol increases the coronary flow at least partly by decreasing the resistance of the coronary bed. In the whole animal, doses of 20 to 90 mgm. have practically no effect on either blood pressure or coronary flow. In contrast to Stoland and Ginsberg's results, Leyko (121) has reported that metrazol does not affect the coronary circulation in the dog heart-lung preparation over a wide range of doses. Lindner and Katz (122) have shown that doses of 100 mgm. of metrazol increase coronary flow in the perfused dog heart in ventricular fibrillation. The increase in flow is slight and lasts about 2 minutes.

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Heparin and dicumarol. Recently Gilbert and Nalefski (59), measuring the coronary sinus outflow of the anesthetized dog with a Morawitz-Zahn cannula, have shown that the sodium salt of heparin increases coronary flow slightly without altering blood pressure or heart rate; the barium salt of heparin has no effect on coronary flow. It is the opinion of Gilbert and Nalefski that soluble barium salts constrict coronary vessels and these authors, therefore, believe it reasonable to assume that the barium salt of heparin has no effect on the coronary circulation because the effect of the barium ion neutralizes the effect of heparin. The disodium salt of dicumarol increases coronary flow markedly and lowers blood pressure slightly. The increase in flow is prolonged and may last longer than that produced by the xanthines. Dicumarol also increases the coronary flow of the beating isolated dog heart whose coronary arteries are perfused under constant pressure. In this preparation, dicumarol also increases the amplitude of ventricular contraction, which effect appears after the increase in coronary flow has begun.

*Digitalis, digitalis bodies and digitalis-like drugs.** Essex, Herrick, Baldes and Mann (42), using a thermostromuhr in unanesthetized dogs, have studied the effect of successive doses of digitalis (digiglusin, Lilly) administered intramuscularly on coronary blood flow and blood pressure. One cat unit of digiglusin was given intramuscularly to a 16 kg. dog twice the first day, once the second day, twice the fourth and once the fifth day of the period of observation. Such doses of digitalis do not alter either coronary blood flow, arterial blood pressure or heart rate. Essex, Herrick and Visscher (46) have studied the effect of intravenous administration of digilanids A, B and C and K-strophanthin on coronary flow and blood pressure in the unanesthetized dog and have concluded that doses just subliminal for nausea and vomiting do not affect either coronary flow or blood pressure. Dearing, Essex, Herrick and Barnes (28) have observed the effect of intravenous administration of digifoline, digalen, digitoxin and lanatoside A on the coronary blood flow and blood pressure of unanesthetized dogs. They have studied the effect of calculated "therapeutic doses" of the preparathe heart rate remains unchanged. In four experiments in which the same authors gave 70 mgm. of nikethamide per kg. intravenously to anesthetized dogs, it was observed that such a dose of coramine increases coronary flow, heart rate and cardiac output and work as well as oxygen consumption of the left ventricle; the arterial blood pressure was lowered and the mechanical efficiency of the left ventricle decreased. Leyko (121) has observed that concentrations of the drug up to 1:50,000 do not affect the heart in the dog heart-lung preparation. With concentrations of 1:10,000 to 1:25,000, the size of the ventricles and the coronary flow increase. Obviously the mechanism responsible for the increase in coronary flow cannot be decided since it is not known whether the increase in coronary flow is due to coronary dilatation or to a decrease in extravascular support. Finally, it must be added that Elek and Katz (39) have observed that nikethamide increases coronary flow of the perfused isolated dog heart in ventricular fibrillation presumably by dilating the coronary bed.

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When experiments on the effect of digitalis and digitalis-like drugs on coronary flow are conducted on anesthetized animals in which coronary sinus outflow is measured, the results are not as clear cut as in the experiments reported above on the unanesthetized animal. This should be no surprise since most of these drugs require some time to achieve their full effect and the circulatory status of an anesthetized dog with chest open and under artificial respiration can hardly be said to remain constant. Gilbert and Fenn (56) measuring the sinus outflow in anesthetized dogs have studied the effect of several preparations of digitalis leaf, ouabain and digitoxin (American and German) on heart rate, blood pressure and coronary flow. The results obtained in different dogs were somewhat variable and one digitalis leaf preparation gave results somewhat different from the results observed with two other digitalis leaf preparations. It seems, however, that 20 to 30 per cent of the lethal dose of most preparations tested produce some decrease in coronary flow that must have been due to an increase in resistance of the coronary bed. These authors think that the increase in resistance of the coronary bed is due to constriction of the coronary vessels. Ginsberg, Stoland and Siler (61) have investigated the effect of fresh digitalis tinctures, digiglusin (Lilly), digalen, digifoline, strophanthin and scillaren on the coronary sinus outflow in the dog denervated heart-lung preparation and in the anesthetized dog. The results were quite variable in different dogs and with different digitalis preparations. However, it seems that in the heart-lung preparation the most consistent effect is a decrease of coronary flow for about 10 minutes, after which the coronary flow generally increases. In the whole animal, similar effects occur but they are still less definite. The authors point out that often the coronary flow of the whole dog is not affected. It seems from the data presented that the decrease in flow, when it occurs, must be due to an increase in resistance of the coronary bed. Page, Wendel, Sheldon and Foltz (137) have recently investigated the effect of ouabain in the anesthetized dog. They determined coronary flow with the nitrous oxide method and also followed arterial blood pressure, heart rate, cardiac output, work, efficiency and oxygen consumption, systemic total peripheral resistance and resistance of the coronary bed. Comparing the data of control experiments and the data collected in experiments in which ouabain was given, these authors conclude that doses of 0.026 mgm. of ouabain per kg. modify significantly only one function studied, cardiac output, which increases. Doses of 0.037 mgm. per kg. raise arterial blood pressure slightly, lower heart rate, increase systemic total peripheral resistance and do not significantly alter other functions. The shortcomings of such a method have been mentioned in the discussion of the xanthines. Recently Bing, Maraist, Damman, Draper, Heimbecker, Daley, Gerard and Calazel (17) have investigated the effect of intraarterial injection of strophanthus preparations on the coronary circulation of unanesthetized normal human beings and of patients with cardiac failure. The coronary flow was estimated with the nitrous oxide method and the cardiac output by the direct Fick method. These authors have found that in normal persons, strophanthus decreases the output, work and efficiency of the left ventricle whereas coronary blood flow, mean arterial blood pressure, coronary oxygen arterio-venous difference and cardiac oxygen consumption are unchanged. In cardiac failure, strophanthus increases the output, work and efficiency of the left ventricle whereas coronary flow and oxygen arterio-venous difference are unchanged. Bodo (18) has shown in the dog heart-lung preparation that 0.0025 mgm. of g-strophanthin per 100 cc. of blood, as well as digitalis tincture and digitalis infusion in equivalent doses, reduces the size of the heart; arterial pressure and cardiac output and work remain constant and coronary flow is slightly increased. The increase in coronary flow as well as the decrease in the size of the ventricles does not appear immediately. The increase in coronary flow while the blood pressure remains unchanged must be due to a decrease in resistance of the coronary bed. Since the size of the ventricles decreases, it seems probable that the decrease in the resistance of the coronary bed is due to coronary dilatation. Lindner and Katz (122), investigating the effect of k-strophanthin, digifolin and ouabain on the coronary circulation of the perfused dog heart in ventricular fibrillation, have reported sometimes an increase in coronary flow, presumably due to dilatation of the coronary bed, and sometimes a decrease in flow, presumably due to constriction. From their clinical observations, Fenn and Gilbert (51) believe that digitalis in therapeutic doses can induce anginal pain in human beings by constricting the coronary arteries. On the other hand, Gold (62, 64) is of a different opinion because, in a large series of patients suffering from effort angina due to coronary sclerosis without cardiac failure, digitalization did not seem to influence either the frequency of pain or the capacity for work.

In conclusion, it seems that in the unanesthetized dog therapeutic doses of digitalis, digitalis bodies and digitalis-like drugs do not modify the coronary flow very much, if at all. Large doses may decrease the coronary flow. The studies made in acute experiments on anesthetized animals which have undergone rather extensive operative procedures have yielded conflicting data, as have studies made on the heart-lung preparation or similar preparations.

Quinidine. Bodo (18) has shown in the dog heart-lung preparation that 0.0066 to 0.01 gram quinidine sulfate increases the diastolic size of the heart but, everything else remaining constant, it does not alter the coronary circulation. In very large doses, however, the drug causes an increase, then a decrease in coronary flow, interpreted by Bodo as due to dilatation followed by constriction of the coronary vessels. Kountz (114) as well as Elek and Katz (39) have observed that quinidine does not affect the coronary flow either in the revived human heart perfused by the Langendorff method (114) or in the perfused dog heart in ventricular fibrillation (39). Massive doses, however, produce coronary dilatation (39).

Morphine. Kountz (114) has observed that morphine increases the coronary flow of the revived human heart perfused by the Langendorff method, but since the doses used depressed the amplitude of ventricular contraction this increase in flow may well have been due to a decrease in extravascular support. Elek and Katz (39) have reported that 0.8 to 1.6 mgm. of morphine sulfate increase the coronary flow in the perfused fibrillating dog ventricles, presumably by dilating the coronary bed. Van Egmond (163) using the Langendorff perfusion technic in cats reported that morphine does not modify coronary flow. In the whole anesthetized dog Wégria, Ward, Frank, Dreyfuss, Brown and Hutchinson (183) have found that 5 to 10 mgm. of morphine sulfate given intravenously to dogs weighing between 11 and 27 kg. either do not affect blood pressure and coronary flow or more often produce a fall in blood pressure and coronary flow. During the recovery from the effect of the drug in those experiments in which morphine has lowered blood pressure and coronary flow, evidence of an increase in resistance of the coronary bed is observed more often than a decrease.

Sodium chloride, calcium chloride, calcium gluconate, potassium chloride, magnesium sulfate. Katz and Lindner (106) have studied the effect of an excess of Na, K and Ca ions on the coronary flow of perfused fibrillating dog ventricles. When the concentration of sodium in the defibrinated blood used for the perfusion is increased to 1.2 to 2.75 times the normal concentration by the addition of sodium chloride, a definite coronary dilatation results; it is of short duration, lasting 1 to 4 minutes. Concentrations of calcium 1.3 to 24 times the control concentration, produced by the addition of calcium chloride, cause a more marked coronary dilatation than an increase in the sodium concentration, the dilatation generally lasting 3 to 15 minutes. Calcium gluconate, however, has no effect or induces only a very slight dilatation of the coronary bed (122). When the concentration of potassium reaches a level of 1.04 to 1.5 times its normal concentration in defibrinated blood after addition of potassium chloride, coronary dilatation results. Concentrations of potassium between 1.55 and 1.66 times the normal concentration cause either coronary dilatation or constriction. With concentrations of 1.66 to 2.5 times the normal concentration, marked coronary constriction is observed. Katz and Lindner (106) point out that even a marked change in the character of the ventricular fibrillation induced by an excess of calcium or potassium does not seem to affect coronary flow in their preparation in which the cavities of the heart are open to atmospheric pressure. Magnesium sulfate always causes a marked coronary dilatation as shown by Elek and Katz (39) on the same type of preparation.

Atropine. Essex, Wégria, Herrick and Mann (47), using the thermostromuhr, have shown that atropine administered intravenously markedly increases heart rate and coronary flow in the unanesthetized dog for 45 minutes or longer. Wégria, Essex, Herrick and Mann (175) have observed that atropine increases the coronary flow of both left and right coronary arteries of the anesthetized dog. The heart rate increases; the blood pressure is not affected or is very slightly increased. The coronary flow reaches its peak within a few minutes and remains maximally increased for as long as one hour. The increase in flow is due mostly, and in most experiments exclusively, to a decrease in resistance of the coronary bed. It is probable that the effect of atropine is similar to cutting both vagi, which procedure has been shown to increase coronary flow in the innervated dog heart-lung preparation (13) in the absence of any changes in heart rate, blood pressure or cardiac output. Whether in the whole animal other changes, such as an increase in cardiac output and work, occur after administration of atropine is not known. Essex, Herrick, Mann and Baldes (45) have shown that atropine increases heart rate and coronary flow in the unanesthetized dog and in the unanesthetized dog with a bilateral sympathetic ganglionectomy from T8 or T9 upward to include the stellate ganglia. Atropine, however, is without effect on heart rate, blood pressure and coronary flow after bilateral cervical vagotomy. Kountz (114) has observed that atropine increases heart rate and diminishes coronary flow in the revived beating human heart perfused by the Langendorff method; atropine also decreases coronary flow in the human heart in standstill.

Neostigmine. Mendez and Ravin (129) have shown in the dog heart-lung preparation that neostigmine decreases slightly the coronary sinus outflow measured with a Morawitz-Zahn cannula.

Pilocarpine. Pilocarpine is said to increase coronary flow of the revived beating human heart perfused by the Langendorff technic as well as the human heart in standstill (114).

Ethyl alcohol. Although alcohol has been recommended by numerous authors for the therapy of angina pectoris, there are few critical experimental or clinical data on the effect of alcohol on the coronary circulation. Dixon (31) reports that concentrations of alcohol of 0.1 to 0.2 per cent dilate the coronary vessels, whereas stronger concentrations (1 to 2 per cent) first constrict and then dilate the coronary vessels of the cat heart in a Langendorff preparation. Sulzer (162), measuring coronary sinus outflow in the dog heart-lung preparation, has observed that concentrations of 0.1 to 0.2 per cent of alcohol in the blood always decrease the coronary flow and increase the diastolic size of the heart, everything else remaining constant. Whether the decrease in coronary flow is due to coronary constriction remains to be established. Recently, Russek, Naegele and Regan (144), studying the effect of alcohol given orally in patients with angina pectoris due to coronary sclerosis, have made the observation that alcohol, although as effective as nitroglycerin in preventing the anginal pain induced by a standard exercise test, fails to prevent the electrocardiographic changes observed during control exercise tests; nitroglycerin does prevent such changes. These authors conclude that alcohol acts mainly by raising the threshold for pain.

Pitressin. There is no disagreement about the effect of pitressin on the coronary circulation. In the anesthetized (175) and the unanesthetized (47) dog, it has been shown with the thermostromuhr that 0.5 to 4 pressor units of pitressin given intravenously decrease the coronary flow very markedly for as long as 45 minutes whereas the blood pressure sometimes decreases, sometimes increases and sometimes first increases and then decreases. The heart rate is always decreased. There is no doubt that pitressin decreases coronary flow by increasing resistance of the coronary bed. Green, Wégria and Boyer (74) have observed the same results with the orifice meter in the anesthetized dog and they have concluded that pitressin is a powerful constrictor of the coronary bed. Bodo (18) has shown in the dog heart-lung preparation that pituitrin decreases coronary flow and produces dilatation of the heart, everything else remaining unchanged. The effect of pituitrin on the coronary flow in the dog heart-lung preparation has been confirmed by Narayana (134). Katz, Lindner, Weinstein, Abramson and Jochim (108) have demonstrated that pitressin produces a marked constriction of the coronary bed in the perfused dog heart in ventricular fibrillation. In the revived human heart perfused by the Langendorff method and in the arrested human heart, pituitrin generally decreases the coronary flow although it sometimes increases it at the end of an experiment and in dilated hearts; the difference in the effect observed is probably due to a difference in the effect of the drug on the myocardium (114, 115, 116). It has indeed been observed that, at the beginning of an experiment, pituitrin modifies amplitude of ventricular contraction very little and decreases heart rate, whereas it increases amplitude of ventricular contraction and heart rate in those preparations in which it increases coronary flow (114–116). Although it seems certain that pitressin decreases coronary flow by constricting the coronary bed, a study of the effect of the drug simultaneously on blood pressure, coronary flow and cardiac output, work and metabolism is still to be done.

V. CONCLUDING REMARKS

After a résumé of the technics generally used for the study of the coronary circulation and a short discussion of the main features of the physiology of the coronary circulation, the effect of drugs on the coronary circulation has been reviewed. With apology for his errors of omission and commission, the reviewer hopes to have pointed out that to gain complete knowledge, as previously defined, of the effect of a drug on the coronary circulation it is mandatory to investigate the effect of that drug not only on simple, very artificial preparations but also on more complicated preparations including ultimately unanesthetized healthy and sick human beings. It hardly needs to be said that a great deal remains to be done.

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and sometimes first increases and then decreases. The heart rate is always decreased. There is no doubt that pitressin decreases coronary flow by increasing resistance of the coronary bed. Green, Wégria and Boyer (74) have observed the same results with the orifice meter in the anesthetized dog and they have concluded that pitressin is a powerful constrictor of the coronary bed. Bodo (18) has shown in the dog heart-lung preparation that pituitrin decreases coronary flow and produces dilatation of the heart, everything else remaining unchanged. The effect of pituitrin on the coronary flow in the dog heart-lung preparation has been confirmed by Narayana (134). Katz, Lindner, Weinstein, Abramson and Jochim (108) have demonstrated that pitressin produces a marked constriction of the coronary bed in the perfused dog heart in ventricular fibrillation. In the revived human heart perfused by the Langendorff method and in the arrested human heart, pituitrin generally decreases the coronary flow although it sometimes increases it at the end of an experiment and in dilated hearts; the difference in the effect observed is probably due to a difference in the effect of the drug on the myocardium (114, 115, 116). It has indeed been observed that, at the beginning of an experiment, pituitrin modifies amplitude of ventricular contraction very little and decreases heart rate, whereas it increases amplitude of ventricular contraction and heart rate in those preparations in which it increases coronary flow (114–116). Although it seems certain that pitressin decreases coronary flow by constricting the coronary bed, a study of the effect of the drug simultaneously on blood pressure, coronary flow and cardiac output, work and metabolism is still to be done.

V. CONCLUDING REMARKS

After a résumé of the technics generally used for the study of the coronary circulation and a short discussion of the main features of the physiology of the coronary circulation, the effect of drugs on the coronary circulation has been reviewed. With apology for his errors of omission and commission, the reviewer hopes to have pointed out that to gain complete knowledge, as previously defined, of the effect of a drug on the coronary circulation it is mandatory to investigate the effect of that drug not only on simple, very artificial preparations but also on more complicated preparations including ultimately unanesthetized healthy and sick human beings. It hardly needs to be said that a great deal remains to be done.

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A Manual of the

ASPERGILLI

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